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## ENTOMON

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## Morphometry of stingless bees (Hymenoptera: Apidae: Meliponini) of the genus *Lisotrigona* indicates presence of more than one species in India

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**ABSTRACT:** Morphometry of 53 stingless bees of the genus *Lisotrigona* collected from seven places in India by using 36 morphological parameters was studied. The data set also included morphometry data of primary types of *L. cacciae*, *L. chandrai* and *L. rewanai* for comparison and was subjected to Factor and Canonical Discriminant analysis. All the bees collected from seven places formed two distinct clusters in the Factor analysis and five clusters in Canonical Discriminant analysis. In both the methods of analysis primary types of *L. cacciae*, *L. chandrai* and *L. rewanai* were placed well separated from each other as well as from other bees. The bees from seven places also differed from the three known species in morphometry and ratios of length and width of parts of the body. Based on these results it is concluded that Indian stingless bees of the genus *Lisotrigona* consists of more than one species besides *L. cacciae*. The action of synonymizing *L. mohandasi*, *L. chandrai* and *L. rewanai* with *L. cacciae* appears arbitrary; these three species should be considered valid until supported by male genital morphology or molecular characters.

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**KEYWORDS:** Morphological parameters, *Lisotrigona* spp., factor analysis, canonical discriminant analysis

### INTRODUCTION

Indian stingless bees (Hymenoptera: Apidae: Meliponini) belong to three genera *Tetragonula* Moure, 1961, *Lepidotrigona* Schwarz, 1939 and *Lisotrigona* Moure, 1961 (Rasmussen, 2013; Viraktamath *et al.*, 2020). The genus *Lisotrigona* includes tiny stingless bees (generally < 3 mm in length) and is quite rare. The genus is represented with four species in India viz. *L. cacciae* (Nurse, 1907), *L. mohandasi* Jobiraj and Narendran, 2004,

*L. chandrai* Viraktamath and Sajan Jose, 2017 and *L. rewanai* Viraktamath and Sajan Jose, 2017 distributed in Madhya Pradesh, Kerala and Maharashtra. Of the four species, three species (*L. cacciae*, *L. mohandasi* and *L. rewanai*) are described based on the museum specimens while one species (*L. chandrai*) based on the detailed studies of males, females, queen and nest structure. No information is available on males, queens, nest structure and nesting habits about the other three species. Females of stingless bees are remarkably

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similar with very weak diagnostic characters. However, males and their genitalia have diagnostic characteristics which are species specific (Schwarz, 1939; Sakagami, 1978; Rasmussen, 2013; Attasopa *et al.*, 2018). Hence Rasmussen (2013) and Attasopa *et al.* (2018) emphasized to make all out efforts to collect males and to include descriptions of both males and females while proposing new taxa.

As the diversity of Indian stingless bees is not fully known (Rasmussen, 2013), an intensive collection of stingless bees by surveying different parts of India was carried out. During our survey, we found feral colonies of *Lisotrigona* and collected series of samples of these bees from Maharashtra, Chhattisgarh and Mizoram. Recently Rasmussen *et al.*, (2017) synonymized *L. mohandasi*, *L. revanai* and *L. chandrai* with *L. cacciae* without giving justification in spite of the fact that *L. chandrai* is described based on series of males and females. Since morphometry is one of the important tools to identify and delineate species in Meliponini which includes several cryptic and species complexes (Moure, 1961; Sakagami, 1978; Franco *et al.*, 2015; Halcroft *et al.*, 2015), an exhaustive study on the morphometry of *Lisotrigona* bees was taken up to find - whether the genus *Lisotrigona* includes only one species (*L. cacciae*) or more in India?

## MATERIALS AND METHODS

During our survey to collect stingless bees from different parts of India, we collected *Lisotrigona* bees in six places namely Darbha (18.85° N, 81.8689° E), Bhanupratappur (20.30° N, 81.07° E), Barnawapara (20.45° N, 81.97° E) (Chhattisgarh), Yavatmal (20.38° N, 78.13° E), Umred (20.84° N, 79.32° E) (Maharashtra) and Thenzawl (23.29° N, 92.75° E) (Mizoram) during 2019-2020 from their foraging sources. However, in Darbha and Barnawapara the samples were collected directly from their colonies. All the samples were collected in a specimen tube containing about 1 ml ethyl acetate absorbed in a cotton wad. Later the bees were transferred to a vial containing 95 per cent ethyl alcohol and labeled indicating the place and date of collection. All the samples were examined

in the Systematic laboratory at the Department of Entomology, University of Agricultural Sciences, Bengaluru, under a Leica stereoscopic microscope (Model: M205C) to confirm the identity of the genus by using key characters enumerated by Rasmussen (2013). There were altogether 53 bees from seven places in the studies, including five bees of *Lisotrigona* found in the old collection of the Department of Entomology, UAS Bengaluru and Zoological Survey of India, Kolkata collected in Raipur (Chhattisgarh).

Thirty-six morphological parameters (modified from Sakagami, 1978; Rasmussen, 2013) were selected for morphometry studies (Table 1). The parameters that included various body parts of the head, thorax and abdomen were measured under the Jenco stereoscopic binocular microscope (Model: ZM-H-602) fitted with an ocular micrometer. The number of hamuli on the right-wing were counted. All the measurements were expressed in millimeters. Mean and standard deviation was calculated for each parameter. Ratios of measurement of different parts of the body as used by Sakagami (1978) were calculated. We also included morphometry data of primary type specimens of *L. chandrai*, *L. revanai* (Viraktamath and Sajan Jose, 2017) and *L. cacciae* (Rasmussen, 2013) in the analysis for comparison. All the data were subjected to log<sub>10</sub> transformation before further analysis (Bookstein, 1985).

Two methods of statistical analysis by using SPSS software (version 16) were adopted to identify discrete morphological groups of bees from these seven places. The data were first subjected to Factor analysis which included analysis of variation, Principal Component analysis (PCA) on a correlation matrix of 30 measured variables. Six variables like DMO, FFI, SFL, TFL, TFW and HAM were not included as the variations among the samples were negligible. Sampling adequacy was verified by Kaiser-Meyer-Olkin measure and Bartlett's test of sphericity. Further, the variables were subjected to Varimax rotation with Kaiser normalization. Morphological groups were identified through a scatter plot by using regression factor score 1 and factor score 2. The second method of analysis was stepwise Canonical Discriminant

Table 1. Morphological parameters selected for morphometry and their abbreviations

Morphological Parameter	Abbreviation
Length of body	BL
Width of head including compound eyes	HW
Length of head	HL
Length of compound eye	EL
Width of compound eye	EW
Upper inter-orbital distance	UIOD
Diameter of median ocellus	DMO
Inter-ocellar distance	IOD
Ocello-ocular distance	OOD
Length of clypeus	CLL
Maximum width of clypeus	CLW
Length of malar space	MSL
Length of scape	SCL
Width of scape	SCW
Length of pedicel + flagellum	FL
Length of first flagellar segment	FFL
Length of second flagellar segment	SFL
Length of third flagellar segment	TFL
Width of third flagellar segment	TFW
Length of mandible	MNL
Width of mandible	MNW
Length of forewing	FWL
Width of forewing	FWW
Length of pterostigma	PTL
Length of marginal cell	MCL
Width of marginal cell	MCW
Diagonal length of forewing	FWD
Number of hamuli	HAM
Length of mesoscutum	MSCL
Maximum width of mesoscutum	MSCW
Length of mesoscutellum	SCTL
Maximum width of mesoscutellum	SCTW
Length of hind tibia	HTL
Width of hind tibia	HTW
Length of hind basitarsus	HBTL
Width of hind basitarsus	HBTW

analysis (CDA) by using all the 36 observed variables. A scatter plot was prepared by using the first two Discriminant Functions to study the clustering of samples.

## RESULTS

Detailed morphometry of the stingless bees from seven places in comparison with three known species is presented in Table 2. *Lisotrigona* bees

from Darbha and Barnawapara were larger with a body length of 3.10 mm as against the body length of 2.78, 2.95 and 2.58 mm in *L. chandrai*, *L. cacciae* and *L. revanai*, respectively. The body length of the bees varied from 2.71 to 3.02 mm in the remaining five places. The head width (including compound eyes) of the bees from Darbha was very close (1.18 mm) to that *L. cacciae* and *L. chandrai* (1.19 mm). While the head width of the bees from other places varied from 1.10 to 1.15 mm. The head length was greater in the bees from Darbha (0.94 mm) while it varied from 0.88 to 0.91 mm in the remaining six places. Head length was 0.77, 1.01, and 0.90 mm in *L. chandrai*, *L. cacciae* and *L. revanai*, respectively. Among the bees collected from seven places, longer forewings (2.65 mm) were recorded in the bees from Bhanupratappur while *L. chandrai* had the longest forewings (2.71 mm) among the three known species. In *L. cacciae* the forewings measured 2.65 mm. Similarly, the diagonal length of the forewing (FWD) was greater in the bees from Bhanupratappur (0.75 mm) while in the known species it was 0.70, 0.73 and 0.70 mm in *L. chandrai*, *L. cacciae* and *L. revanai*, respectively. The lowest diagonal length of the forewing (0.64 mm) was observed in the bees from Barnawapara. The longest hind tibia was recorded in the bees from Darbha (0.89) as compared to *L. chandrai* (0.84 mm), *L. cacciae* (0.86 mm) and *L. revanai* (0.86 mm).

The ratios of different parts of the body of the bees from seven places in comparison with the three known species are presented in Table 3. The ratio of length and width of head (HL/HW) was highest in *L. cacciae* (0.85) and lowest in *L. chandrai* (0.65). In the bees from seven places, the ratio varied from 0.77 to 0.82. On the other hand, the ratio of the length of scape and eye (SCL/EL) was the lowest in *L. cacciae* (0.45) and highest in the bees from Thenzawl (0.59). The ratio of width and length of the hind tibia (HTW/HTL) was higher in the bees from seven places (0.39 to 0.44) compared to the ratio observed in *L. chandrai* (0.39), *L. cacciae* (0.36) and *L. revanai* (0.34). Differences in the ratios of other body parts were not apparent between the freshly collected and the three known species.

Table 2. Morphometry of female stingless bees of the genus *Lisotrigona* from India (Mean in mm  $\pm$  Standard deviation)

No	Parameter/Place	DB N: 10	BPN: 01	BW N: 05	RP N: 04	YM N: 10	UR N: 10	TZ N: 10	<i>L. chandrai</i> *	<i>L. cacciae</i> **	<i>L. revanai</i> *
1	BL	3.10 $\pm$ 0.14	2.85	3.10 $\pm$ 0.19	3.02 $\pm$ 0.02	2.92 $\pm$ 0.21	2.74 $\pm$ 0.10	2.71 $\pm$ 0.06	2.78	2.95	2.58
2	HW	1.18 $\pm$ 0.02	1.15	1.14 $\pm$ 0.01	1.10 $\pm$ 0.00	1.11 $\pm$ 0.03	1.10 $\pm$ 0.01	1.15 $\pm$ 0.04	1.19	1.19	1.14
3	HL	0.94 $\pm$ 0.01	0.88	0.91 $\pm$ 0.02	0.88 $\pm$ 0.02	0.91 $\pm$ 0.02	0.89 $\pm$ 0.01	0.89 $\pm$ 0.03	0.77	1.01	0.90
4	EL	0.84 $\pm$ 0.02	0.85	0.82 $\pm$ 0.02	0.81 $\pm$ 0.01	0.81 $\pm$ 0.04	0.77 $\pm$ 0.02	0.81 $\pm$ 0.03	0.83	0.83	0.88
5	EW	0.34 $\pm$ 0.01	0.33	0.29 $\pm$ 0.01	0.28 $\pm$ 0.01	0.30 $\pm$ 0.01	0.28 $\pm$ 0.01	0.31 $\pm$ 0.02	0.35	0.33	0.28
6	UIOD	0.75 $\pm$ 0.02	0.75	0.73 $\pm$ 0.02	0.74 $\pm$ 0.03	0.72 $\pm$ 0.02	0.73 $\pm$ 0.02	0.75 $\pm$ 0.02	0.76	0.75	0.78
7	DMO	0.11 $\pm$ 0.01	0.12	0.11 $\pm$ 0.01	0.10 $\pm$ 0.01	0.10 $\pm$ 0.00	0.12 $\pm$ 0.00	0.10 $\pm$ 0.01	0.09	0.11	0.11
8	IOD	0.28 $\pm$ 0.00	0.28	0.27 $\pm$ 0.01	0.27 $\pm$ 0.01	0.26 $\pm$ 0.01	0.25 $\pm$ 0.01	0.27 $\pm$ 0.01	0.26	0.27	0.29
9	OOD	0.14 $\pm$ 0.01	0.14	0.14 $\pm$ 0.01	0.14 $\pm$ 0.01	0.15 $\pm$ 0.01	0.14 $\pm$ 0.01	0.16 $\pm$ 0.01	0.18	0.18	0.18
10	CLL	0.26 $\pm$ 0.01	0.25	0.24 $\pm$ 0.01	0.25 $\pm$ 0.00	0.25 $\pm$ 0.01	0.25 $\pm$ 0.01	0.24 $\pm$ 0.01	0.25	0.24	0.28
11	CLW	0.55 $\pm$ 0.01	0.55	0.57 $\pm$ 0.01	0.52 $\pm$ 0.01	0.49 $\pm$ 0.01	0.51 $\pm$ 0.02	0.56 $\pm$ 0.02	0.58	0.42	0.39
12	MSL	0.01 $\pm$ 0.00	0.01	0.02 $\pm$ 0.00	0.05 $\pm$ 0.00	0.01 $\pm$ 0.00	0.02 $\pm$ 0.00	0.04 $\pm$ 0.01	0.03	0.02	0.03
13	SCL	0.42 $\pm$ 0.01	0.45	0.41 $\pm$ 0.01	0.42 $\pm$ 0.02	0.41 $\pm$ 0.01	0.39 $\pm$ 0.01	0.41 $\pm$ 0.01	0.49	0.37	0.47
14	SCW	0.08 $\pm$ 0.00	0.08	0.08 $\pm$ 0.00	0.10 $\pm$ 0.01	0.07 $\pm$ 0.01	0.07 $\pm$ 0.00	0.07 $\pm$ 0.01	0.08	0.07	0.07
15	FL	0.89 $\pm$ 0.04	0.90	0.92 $\pm$ 0.03	0.81 $\pm$ 0.03	0.85 $\pm$ 0.04	0.87 $\pm$ 0.02	0.85 $\pm$ 0.01	0.92	—	0.91
16	FFL	0.06 $\pm$ 0.00	0.06	0.05 $\pm$ 0.01	0.06 $\pm$ 0.01	0.06 $\pm$ 0.01	0.06 $\pm$ 0.01	0.06 $\pm$ 0.01	0.08	0.06	0.08
17	SFL	0.07 $\pm$ 0.00	0.07	0.06 $\pm$ 0.01	0.09 $\pm$ 0.01	0.06 $\pm$ 0.00	0.06 $\pm$ 0.01	0.05 $\pm$ 0.00	0.07	0.06	0.08
18	TFL	0.08 $\pm$ 0.01	0.08	0.06 $\pm$ 0.01	0.08 $\pm$ 0.01	0.06 $\pm$ 0.00	0.06 $\pm$ 0.01	0.05 $\pm$ 0.00	0.07	0.07	0.09
19	TFW	0.10 $\pm$ 0.00	0.10	0.26 $\pm$ 0.03	0.09 $\pm$ 0.01	0.10 $\pm$ 0.00	0.10 $\pm$ 0.00	0.09 $\pm$ 0.01	0.10	0.11	0.10
20	MNL	0.43 $\pm$ 0.02	0.50	0.42 $\pm$ 0.02	0.43 $\pm$ 0.01	0.43 $\pm$ 0.02	0.44 $\pm$ 0.01	0.47 $\pm$ 0.00	0.48	0.45	0.45
21	MNW	0.18 $\pm$ 0.00	0.22	0.18 $\pm$ 0.01	0.18 $\pm$ 0.01	0.18 $\pm$ 0.00	0.16 $\pm$ 0.01	0.18 $\pm$ 0.00	0.16	0.12	0.16
22	FWL	2.59 $\pm$ 0.05	2.65	2.48 $\pm$ 0.08	2.52 $\pm$ 0.02	2.50 $\pm$ 0.06	2.44 $\pm$ 0.04	2.63 $\pm$ 0.07	2.71	2.65	2.69
23	FWW	0.97 $\pm$ 0.03	0.95	0.83 $\pm$ 0.03	0.91 $\pm$ 0.02	0.87 $\pm$ 0.03	0.90 $\pm$ 0.04	0.94 $\pm$ 0.05	1.00	0.95	1.04
24	PTL	0.45 $\pm$ 0.01	0.45	0.41 $\pm$ 0.01	0.44 $\pm$ 0.01	0.40 $\pm$ 0.01	0.44 $\pm$ 0.01	0.45 $\pm$ 0.01	0.41	0.36	0.40
25	MCL	0.77 $\pm$ 0.02	0.73	0.75 $\pm$ 0.00	0.75 $\pm$ 0.01	0.73 $\pm$ 0.02	0.75 $\pm$ 0.01	0.79 $\pm$ 0.01	0.78	0.89	0.77
26	MCW	0.19 $\pm$ 0.01	0.22	0.21 $\pm$ 0.01	0.18 $\pm$ 0.01	0.18 $\pm$ 0.01	0.20 $\pm$ 0.01	0.20 $\pm$ 0.01	0.18	0.18	0.17
27	FWD	0.74 $\pm$ 0.01	0.75	0.64 $\pm$ 0.01	0.69 $\pm$ 0.01	0.65 $\pm$ 0.02	0.66 $\pm$ 0.02	0.72 $\pm$ 0.02	0.70	0.73	0.70
28	HAM	5.00 $\pm$ 0.00	5.00	5.00 $\pm$ 0.00	5.00 $\pm$ 0.00	5.00 $\pm$ 0.00	5.00 $\pm$ 0.00	5.10 $\pm$ 0.03	5.00	6.00	5.00
29	MSCL	0.77 $\pm$ 0.02	0.75	0.70 $\pm$ 0.01	0.70 $\pm$ 0.04	0.71 $\pm$ 0.02	0.69 $\pm$ 0.02	0.71 $\pm$ 0.01	0.60	0.71	0.60
30	MSCW	0.92 $\pm$ 0.02	0.95	0.88 $\pm$ 0.02	0.87 $\pm$ 0.02	0.88 $\pm$ 0.02	0.88 $\pm$ 0.03	0.91 $\pm$ 0.02	0.90	0.93	0.90
31	SCTL	0.25 $\pm$ 0.01	0.25	0.24 $\pm$ 0.01	0.24 $\pm$ 0.01	0.21 $\pm$ 0.01	0.21 $\pm$ 0.01	0.25 $\pm$ 0.00	0.23	0.23	0.28
32	SCTW	0.75 $\pm$ 0.01	0.75	0.75 $\pm$ 0.04	0.63 $\pm$ 0.02	0.74 $\pm$ 0.03	0.72 $\pm$ 0.02	0.78 $\pm$ 0.04	0.45	0.48	0.64
33	HTL	0.89 $\pm$ 0.02	0.85	0.85 $\pm$ 0.04	0.73 $\pm$ 0.02	0.82 $\pm$ 0.02	0.83 $\pm$ 0.02	0.88 $\pm$ 0.03	0.84	0.86	0.86
34	HTW	0.35 $\pm$ 0.01	0.37	0.34 $\pm$ 0.01	0.32 $\pm$ 0.01	0.32 $\pm$ 0.01	0.34 $\pm$ 0.01	0.35 $\pm$ 0.01	0.33	0.31	0.29
35	HBTL	0.44 $\pm$ 0.01	0.45	0.46 $\pm$ 0.02	0.42 $\pm$ 0.03	0.45 $\pm$ 0.01	0.45 $\pm$ 0.00	0.45 $\pm$ 0.01	0.42	0.35	0.38
36	HBTW	0.21 $\pm$ 0.01	0.23	0.23 $\pm$ 0.02	0.21 $\pm$ 0.01	0.20 $\pm$ 0.03	0.21 $\pm$ 0.01	0.22 $\pm$ 0.01	0.23	0.18	0.21

DB: Darbha; BH: Bhanupratappur; BW: Barnawapara; RP: Raipur; YM: Yavatmal; UR: Umred; TZ:Thenzawl

*L. cacciae*\*\* - Primary Type; *L. chandrai*\*; *L. cacciae*\*\* - *L. revanai*\* (N: 10)

In the Canonical Discriminant analysis (CDA), eight functions were extracted with Eigen value more than 1.00 which together influenced 99.3 percent variation. Wilk's lambda values for all these eight functions were significant ( $<0.001$ ). In the first function morphological parameters like MSCL, UIOD, FL, EW, HW, HBTL and MNW had higher





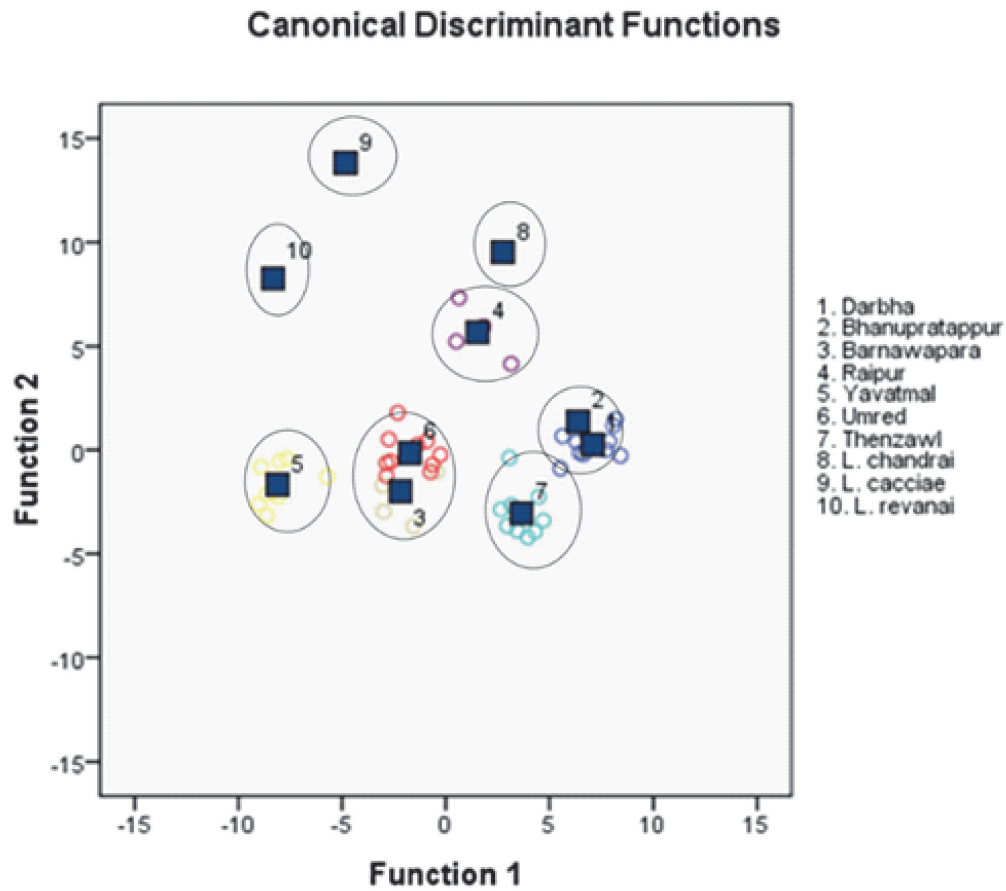


Fig 2. Discriminant analysis scatter plot showing clusters of female stingless bees of the genus *Lisotrigona* from India

Table 3. Ratios of different parts of the body of stingless bees of the genus *Lisotrigona* from India

SN	Parts of body	DB	BP	BW	RP	YM	UR	TZ	<i>L. chandrai</i>	<i>L. cacciae</i>	<i>L. revanai</i>
1	HL/HW	0.79	0.77	0.79	0.80	0.82	0.81	0.77	0.65	0.85	0.79
2	EL/UIOD	1.12	1.13	1.12	1.19	1.13	1.05	1.08	1.09	1.11	1.13
3	IOD/UIOD	0.37	0.37	0.37	0.36	0.36	0.34	0.36	0.34	0.36	0.37
4	SCL/EL	0.50	0.53	0.50	0.52	0.51	0.51	0.51	0.59	0.45	0.53
5	FWL/FWW	2.67	2.79	2.98	2.77	2.87	2.71	2.79	2.71	2.79	2.59
6	FWD/HW	0.63	0.65	0.56	0.63	0.59	0.60	0.63	0.59	0.61	0.61
7	HTL/HW	0.75	0.74	0.75	0.66	0.75	0.75	0.76	0.71	0.72	0.75
8	HTL/FWD	1.20	1.13	1.33	1.06	1.26	1.26	1.22	1.20	1.18	1.23
9	HTW/HTL	0.39	0.44	0.40	0.44	0.39	0.41	0.40	0.39	0.36	0.34
10	HBTW/HTW	0.48	0.51	0.50	0.50	0.44	0.47	0.49	0.55	0.51	0.55

DB: Darbha; BH: Bhanupratappur; BW: Barnawapara; RP: Raipur; YM: Yavatmal; UR: Umred; TZ: Thenzawl

Table 4. Comparison of morphometry of three recently described species of *Lisotrigona* from India with that of *Lisotrigona cacciae*

Parameter	<i>Lisotrigona mohandasi</i> *	<i>Lisotrigona revanai</i> **	<i>Lisotrigona chandrai</i> **	Lectotype - <i>Lisotrigona cacciae</i> *** (India)	<i>Lisotrigona cacciae</i> - southeast Asia****
Length of body	3.00	2.58	2.78	2.95	3.06-3.64
Width of head including eyes	1.28	1.14	1.19	1.19	1.0-1.25
Ratio between length and width of head	2.34	2.26	2.34	2.48	--
Length of head (Clypeal apex-posterior margin of vertex)	--	0.99	0.77	1.01	0.98-1.13
Length of compound eye	--	0.88	0.83	0.83	0.80-0.90
Width of compound eye	--	0.28	0.35	0.33	--
Upper interorbital distance	--	0.78	0.76	0.75	0.73-0.80
Maximum interorbital distance	--	0.82	0.79	0.85	0.78-0.88
Lower interorbital distance	--	0.68	0.61	0.65	0.63-0.70
Diameter of median ocellus	--	0.11	0.09	0.11	--
Interocellar distance	--	0.29	0.26	0.27	0.21-0.28
Ocello-orbital distance	0.20	0.18	0.18	0.18	0.13-0.23
Length of clypeus	--	0.28	0.25	0.24	--
Maximum width of clypeus	--	0.39	0.38	0.42	--
Inter-tentorial distance	--	0.28	0.26	0.36	--
Clypeo-ocellar distance	--	0.74	0.68	0.06	--
Length of malar space	--	0.03	0.03	0.02	--
Length of antennae	--	1.38	1.41	--	--
Length of scape	0.20	0.47	0.49	0.37	0.33-0.40
Diameter of scape	--	0.07	0.08	0.07	--
Ratio between length and diameter of scape	2.86	6.71	6.13	5.29	--
Diameter of third flagellomere	--	0.10	0.10	0.11	--
Length of first flagellomere	--	0.08	0.08	0.06	0.08
Length of second flagellomere	--	0.08	0.07	0.06	0.08
Length of third flagellomere	--	0.09	0.07	0.07	--
Length of mandible	--	0.45	0.48	0.45	--
Width of mandible	--	0.16	0.16	0.12	--
Ratio between length and width of mandible	--	2.81	3.00	3.75	--
Parameter	<i>Lisotrigona mohandasi</i> *	<i>Lisotrigona revanai</i> **	<i>Lisotrigona chandrai</i> **	Lectotype of <i>Lisotrigona cacciae</i> *** (India)	<i>Lisotrigona cacciae</i> from southeast Asia****
Length of proboscis	--	1.19	1.18	--	--
Length of forewing excluding tegula	--	2.49	2.51	2.37	--
Length of forewing including tegula	2.60	2.69	2.71	2.65	--
Width of forewing	--	1.04	1.00	0.95	--
Ratio of forewing length including tegula and width of forewing	--	2.59	2.71	2.79	--

Length of pterostigma	--	0.40	0.41	0.36	--
Width of pterostigma	--	0.10	0.10	0.12	--
Ratio between length and width of pterostigma	--	4.00	4.10	3.00	--
Length of marginal cell	--	0.77	0.78	0.89	--
Width of marginal cell	--	0.17	0.18	0.18	--
Ratio of length and width of marginal cell	--	4.53	4.33	4.94	--
Hamuli	--	5.40	5.00	6	--
Length of mesoscutum	--	0.60	0.60	0.71	--
Width of mesoscutum	--	0.90	0.90	0.93	--
Length of scutellum	--	0.28	0.23	0.23	--
Width of scutellum	--	0.64	0.45	0.48	--
Length of femur III	--	0.66	0.68	--	--
Length of tibia III	--	0.86	0.84	0.86	--
Width of tibia III	--	0.29	0.33	0.31	--
Ratio of length of tibia III and width	--	2.96	2.54	2.77	--
Length of basitarsus III	--	0.38	0.42	0.35	--
Width of basitarsus III	--	0.21	0.23	0.18	--
Ratio of length and width of basitarsus III	--	1.81	1.83	1.94	--
Ratio of length of basitarsus III and head width	--	0.33	0.35	0.29	--
Width of tergum III	--	--	--	1.05	--
Length of hairs on clypeus	--	0.02	0.02	<0.01	--
Length of hairs on frons	--	0.04	0.04	0.02	--
Length of hairs on the vertex	--	--	--	0.04	--
Length of hairs on scutellum apex	--	0.12	0.09	0.13	--

\* Data from Jobiraj and Narendran (2004)

\*\* Data from Viraktamath and Sajan Jose (2017)

\*\*\* Data from Lectotype (Rasmussen, 2013)

\*\*\*\*Data from Engel (2000)

loading factors ranging from 0.211 to 0.353 which accounted for 43.8 percent variation. Results of CDA scatter plot drawn by using Canonical Discriminant function 1 and 2 segregated the bees from seven places into the following five distinct clusters.

Cluster 1. Bees from Darbha + Bhanupratappur

Cluster 2. Bees from Barnawapara + Umred

Cluster 3. Bees from Raipur

Cluster 4. Bees from Yavatmal

Cluster 5. Bees from Thenzawl

As observed in PCA, *L. chandrai*, *L. cacciae* and *L. revanai* were placed well separated from each other confirming their distinctiveness from other bees (Fig. 2). Classification of the bees into various clusters by the CDA indicated that 98.10 percent of original grouped cases and 90.60 percent of cross-validated grouped cases were correctly classified.

## DISCUSSION

Stingless bees of the genus *Lisotrigona* are distributed in India, Sri Lanka and southeast Asia. The genus altogether includes six species namely



*L. carpenteri* Engel 2000, *L. furva* Engel 2000, *L. cacciae*, *L. mohandasi*, *L. chandrai* and *L. revanai*. Among these, *L. carpenteri* is the most distinctive species with yellow maculation on the face and a body length > 4.00 mm (Engel, 2000). However, *L. furva* and *L. cacciae* which are sympatric, were difficult to identify because of overlapping coloration, pilosity and morphometry (Michener, 2007). *L. cacciae* is the only species with a wide range of distribution occurring in India, Sri Lanka, Malaysia, Thailand, Cambodia and Vietnam (Engel, 2000; Michener, 2007; Karunaratne *et al.*, 2017). Only two species (*L. furva* and *L. chandrai*) are known with both females and males and the male genitalia are quite distinct from each other (Michener, 2007; Viraktamath and Sajan Jose, 2017). *L. cacciae* is the first species of the genus described by Nurse in 1907 with Hoshangabad, India as the type locality (Rasmussen 2013). But the males of this species are unknown so far. The body length of the primary type of *L. cacciae* from India is 2.95 mm (Rasmussen, 2013) but the bees from southeast Asia measure from 3.06 to 3.64 mm (Engel 2000). Three species described from India have a body length of 3.00 mm (*L. mohandasi*), 2.58 mm (*L. revanai*) and 2.78 mm (*L. chandrai*) (Jobiraj and Narendran, 2004; Viraktamath and Sajan Jose, 2017). Similarly, the head width, head length, forewing length, forewing diagonal length and hind tibial length differ among these species. In spite of these and other differences (Table 4), Rasmussen *et al.* (2017) synonymized *L. mohandasi*, *L. chandrai* and *L. revanai* with *L. cacciae* without giving justification. The decision was probably based on similarities in coloration among these species. The variation among these by selecting 36 morphological parameters and compared them with the primary types of *L. cacciae*, *L. chandrai* and *L. revanai*, indicated that results of CDA were more robust than those of Factor analysis. In both the methods of analysis freshly collected bee samples and all the three known species segregated into distinct clusters. In Factor analysis bees from seven places formed two distinct clusters while in CDA they formed five clusters. All three known species (*L. cacciae*,

*L. chandrai* and *L. revanai*) were placed well separated from each other as well as from other bees in both Factor and CDA. These results indicate that more than one species of *Lisotrigona* (other than *L. cacciae*) exist in India. There would have been a single compact cluster in both Factor and CDA if there was only one species. Hence, the action of Rasmussen *et al.* (2017) needs to be considered as arbitrary until supported by male genital morphology or molecular characters.

There is a need to collect males of *L. cacciae* from the areas of its currently known distribution and make comparative studies of male genitalia and sternal characters as they have clear diagnostic characters as reported by Schwarz (1939), Sakagami (1978), Rasmussen (2013) and Attasopa *et al.* (2018). In addition, molecular sequences of *L. cacciae* collected from different places need to be studied and compared. Until these results are available, it is proposed that all the described species of *Lisotrigona* from India (*L. mohandasi*, *L. chandrai* and *L. revanai*) should be treated as valid species.

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## Ovicidal, larvicidal and pupicidal activity of *Nelumbo nucifera* Gaertn against the filarial vector *Culex quinquefasciatus* Say (Diptera: Culicidae)

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**ABSTRACT:** In the current investigation, different solvent leaf extracts of *Nelumbo nucifera* were tested for their mosquitocidal potential against the filarial vector *Culex quinquefasciatus*. Further, bioactive compounds of the crude leaf extracts of *N. nucifera* were identified using GC-MS analysis. The benzene leaf extracts of *N. nucifera* showed significant egg, larval and pupal mortality at concentrations of 62.5, 125, 250 and 500 ppm, respectively. Mortality of ovicidal, larvicidal and pupicidal effect at 500 ppm at 48 h period were 89, 38 and 67 per cent with LC<sub>50</sub> values 4.247, 6.694 and 4.975 and LC<sub>90</sub> values 5.881, 9.628, 6.565 ppm, respectively. GC-MS profile of the leaf showed seven peaks and the major components identified as nuciferine, steporphine and mecambroline (29.40%). The study confirms *N. nucifera* has significant mosquitocidal effects.

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**KEYWORDS:** *Nelumbo nucifera*, mosquitocidal potential, nuciferine, steporphine, mecambroline

### INTRODUCTION

Among all insect vectors of human diseases, mosquitoes stand out as the most medically important fauna causing significant public health problem. Pathogens and parasites are the causative agents of vector-borne diseases (VBDs) and serve as major cause of death especially in the tropical and subtropical countries (Kovendan *et al.*, 2012; Singh *et al.*, 2014). Mosquitoes act as vectors for maladies such as filariasis, malaria, dengue, chikungunya, yellow fever, West Nile, and Zika viral disease (WHO, 2014, 2016). The control of mosquitoes has become a major public concern all over the world (Islam *et al.*, 2011).

Spread of mosquito-borne diseases and deaths can be controlled by preventing the mosquito larvae from maturing into adults (Al-Doghariri and Elhag, 2003). Control of VBDs using broad-spectrum insecticides caused undesirable side effects (Youdeowei and Service, 1983). India has a vast resource of plants which contain phytochemicals which are virtually an untapped reservoir of pesticides that can be used directly or as templates for synthetic pesticides due to their eco-friendly nature (Sekar, 2010; Okwute, 2012). *Nelumbo nucifera* Gaertn. (Nymphaeaceae) known as Indian lotus or sacred lotus is a hydrophyte that produces individual leaves and flowers directly from the root

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system (Shen Miller *et al.*, 2002). The whole plant has various medical properties such as diuretic, astringent and palliative and finds therapeutic use in the treatment of diarrhea, tissue irritation and homeostasis. *N.nucifera* leaves are used for treatment of hematemesis, epistaxis, hematuria, and metrorrhagia. Several bioactive compounds such as alkaloids, steroids, triterpenoids, flavonoids, glycosides and polyphenols were identified from different parts of the plant (Paudel and Panth, 2015). The current study was undertaken to examine the effects of five different solvent extracts of the leaves of *N. nucifera* against the egg, larvae and pupal stages of *Cx. quinquefasciatus*.

## MATERIALS AND METHODS

### Plant material and extraction procedures:

Fresh and mature leaves of *N. nucifera* were collected thoroughly washed, shade dried and pulverized using a blender. By using a Soxhlet apparatus (Vogel, 1978) about one kg of the pulverized leaves were serially extracted with three liters of the solvents (hexane, benzene, ethyl acetate, methanol and distilled water) to obtain the active compounds. The extracts were filtered and further dried using a rotary evaporator. Stock solution (1 %) was prepared by adding required quantity of acetone to each extract and stored at 4°C until further bioassay (Annie *et al.*, 2015).

**Mosquito culture:** *Cx. quinquefasciatus* immature stages were collected from local water bodies and colonized in the laboratory to obtain the F1 generations. The larvae were maintained in enamel trays at  $27 \pm 2^\circ\text{C}$  and 70 to 80 per cent relative humidity and fed with powdered dog biscuit and yeast in the ratio 3:1 (Annie *et al.*, 2015).

**Ovicidal bioassay:** The leaf extracts were screened for their ovicidal efficacy using conventional WHO susceptibility test method (WHO, 2005). The egg rafts laid by F1 generation of the laboratory colonized mosquitoes were collected. By serial dilution of 1 per cent stock solution, test concentrations (62.5, 125, 250 and 500 ppm) of each of the crude extract was prepared. In all, 20 fresh eggs were separated from the egg raft with the aid of a dissection microscope and

brush and were introduced into 250mL plastic containers containing 200 ml of test concentration and water. For each concentration, five replicates per trial were performed along with appropriate controls. Addition of acetone to distilled water served as treated control, while distilled water alone served as an untreated control. After 48 h the hatchability was calculated (Veni *et al.*, 2017).

**Larvicidal and Pupicidal Bioassay:** The solvent extracts were screened for their efficacy against larval and pupal stages of *Cx. quinquefasciatus* using WHO standard susceptibility test method (WHO, 2005). The required test concentrations (62.5, 125, 250 and 500 ppm) was prepared by serial dilution of 1 per cent stock solution for each crude extract. For the present study 20 IV instar larvae and pupae were collected from laboratory colonized F1 generations mosquitoes and introduced separately into 250ml plastic containers containing 200ml of test concentration and water. For each concentration, five replicates per trial were performed along with treated and untreated controls.

### Gas chromatography mass spectrum (GC-MS)

**Analysis:** Among the solvents evaluated, benzene extract of the leaf of *N. nucifera* showed maximum mosquitocidal activity. GC-MS was performed to identify the phytochemicals responsible for the mosquitocidal activity (Abirami and Rajendran, 2012). The GC-MS analysis was performed using Clarus 500 Perkin – Elmer Gas Chromatograph coupled to Perkin Elmer Turbomass 5.1 spectrometer. The starting temperature of the oven was  $110^\circ\text{C}$ , and maintained for 2 min at this temperature. After the time period of 2 min the oven temperature was raised up to  $280^\circ\text{C}$ , at the rate  $5^\circ\text{C}/\text{min}$ , and subsequently maintained at this temperature for 9 min. Temperature at the injection port was maintained at  $250^\circ\text{C}$  and Helium was used as a carrier gas with a flow rate as one ml/min. Ionization voltage was 70eV. The samples were injected in split mode as 10:1. The individual phytochemicals in the crude extracts were separated by the gas chromatography column and the separated compounds enter the Mass Spectrum (MS) and get ionized. The MS ionizing spectrum

was recorded and compared to the MS spectrum of known compounds within the NIST library. Compound name, retention time, area per cent, molecular formula and molecular weight of the components of the subjected extracts were determined.

**Data analysis:** Mortality for all the three bioassays was calculated (Veni *et al.*, 2017) and Abbott's formula was used when control mortality varied between 5 to 20 per cent (Abbott *et al.*, 1925). Data from all replicates pertaining to ovidical, larvicidal and pupicidal bioassay were pooled for statistical analysis. SPSS software by probit analysis (SPSS, 2009) was used for calculating LC50 and LC90 values. Difference in mortality between concentrations was determined using ANOVA and results with  $P < 0.05$  level were considered to be statistically significant.

## RESULTS AND DISCUSSION

Benzene extracts of the leaves of *N. nucifera* exhibited significant mosquitocidal activity against *Cx. quinquefasciatus*. Ovidical, larvicidal and pupicidal activity at 500ppm after 48 h period was 89, 38 and 67 per cent, with LC50 and LC90 values of 4.247, 6.694, 4.975 and 5.881, 9.628, 6.565 ppm respectively. A direct correlation was observed between the ovidical, larvicidal and pupicidal activity and the extract concentration (62.5 to 500 ppm), the rate of mortality was directly proportional to

the concentration indicating that the extracts exhibited a dose-dependent mortality effect on all the stages. No mortality was noticed in the controls (Table 1 and 2).

Since, the benzene extract of the leaves of *N. nucifera* was found to be more effective, it was further subjected to Gas Chromatography-Mass Spectral analysis to identify the phytoconstituents which might be responsible for their significant mosquitocidal activity. Comparison of the mass spectra of the constituents of the extract when matched with NIST library revealed the presence of seven phytocompounds with their corresponding peaks at different retention time (Fig 1). The Retention Time, peak area (%), molecular formula and molecular weight of the benzene leaf extract of *N. nucifera* were identified (Table 3). The compounds hexadecanoic acid (9.02%), hexadecatrienoic acid, linoleyl alcohol (8.74%), nornuciferine (3.15%), roemerine (4.86), 10 nondecanol (7.44%), oxirane, tetradecyl, tetradecanal (12.00%) were recorded. The major constituents were nuciferine, steporphine, mecambroline (29.40%).

Vector borne disease remains a major cause of mortality worldwide. In recent years, the significance of biological activity of phytocompounds in mosquito eradication programmes has gained importance because of their biodegradable nature. Insecticide-resistant

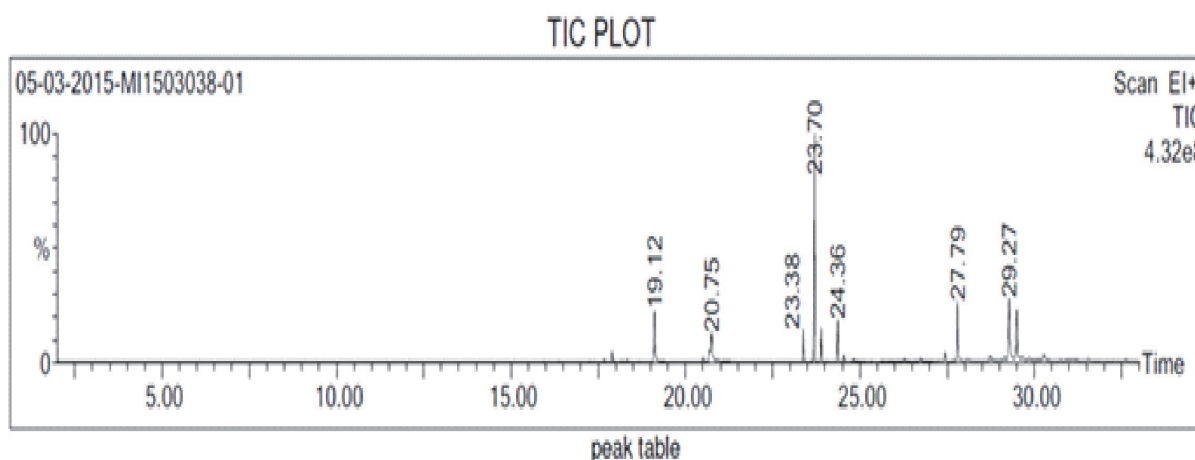


Fig. 1 Chromatogram of benzene extract of *N. nucifera*



Table 1. Ovicidal, larvicidal and pupicidal activity of *N. nucifera* crude leaf extract against *C. quinquefasciatus*

Stages	Solvents	Concentration (ppm)			
		62.5	125	250	500
Ovicidal	Hexane	0.8±1.78 <sup>a</sup> (4.00)	2.2±3.49 <sup>ab</sup> (11.00)	4.2±1.30 <sup>ab</sup> (21.00)	6.0±4.0 <sup>b</sup> (30.00)
	Benzene	4.4±2.30 <sup>b</sup> (22.00)	9.4±1.34 <sup>c</sup> (47.00)	14.2±2.68 <sup>d</sup> (71.00)	17.8±1.48 <sup>c</sup> (89.00)
	Ethyl Acetate	1.6±1.34 <sup>a</sup> (8.00)	5.4±2.60 <sup>b</sup> (27.00)	6.8±2.16 <sup>b</sup> (34.00)	10.2±1.78 <sup>c</sup> (51.00)
	Methanol	1.2±0.83 <sup>ab</sup> (6.00)	2.4±1.51 <sup>bc</sup> (12.00)	2.6±1.34 <sup>bc</sup> (13.00)	3.4±1.34 <sup>c</sup> (17.00)
	Aqueous	2.8±1.92 <sup>ab</sup> (14.00)	4.0±1.87 <sup>bc</sup> (20.00)	6.8±1.30 <sup>cd</sup> (34.00)	8.6±2.60 <sup>d</sup> (43.00)
Larvicidal	Hexane	0.2±0.44 <sup>a</sup> (1.00)	0.8±0.83 <sup>a</sup> (4.00)	1.6±1.81 <sup>ab</sup> (8.00)	3.4±1.51 <sup>b</sup> (17.00)
	Benzene	2.0±1.41 <sup>ab</sup> (10.00)	2.6±1.81 <sup>ab</sup> (13.00)	4.0±1.41 <sup>b</sup> (20.00)	7.6±2.07 <sup>c</sup> (38.00)
	Ethyl Acetate	1.0±1.22 <sup>ab</sup> (5.00)	2.2±0.83 <sup>bc</sup> (11.00)	3.2±1.30 <sup>c</sup> (16.00)	4.0±1.58 <sup>c</sup> (20.00)
	Methanol	0.6±1.34 <sup>a</sup> (3.00)	1.0±1.41 <sup>a</sup> (5.00)	1.6±1.67 <sup>a</sup> (8.00)	2.0±1.0 <sup>a</sup> (10.00)
	Aqueous	0.2±0.44 <sup>a</sup> (1.00)	0.4±0.54 <sup>a</sup> (2.00)	0.6±0.54 <sup>a</sup> (3.00)	1.0±1.0 <sup>a</sup> (5.00)
Pupicidal	Hexane	2.2±0.44 <sup>ab</sup> (11.00)	6.0±4.24 <sup>bc</sup> (30.00)	9.8±5.16 <sup>c</sup> (49.00)	10.6±3.28 <sup>c</sup> (53.00)
	Benzene	5.0±4.58 <sup>ab</sup> (25.00)	6.6±3.28 <sup>b</sup> (33.00)	9.8±4.49 <sup>bc</sup> (49.00)	13.4±1.34 <sup>c</sup> (67.00)
	Ethyl Acetate	0.2±0.44 <sup>a</sup> (1.00)	0.6±0.89 <sup>ab</sup> (3.00)	1.0±1.0 <sup>ab</sup> (5.00)	2.2±1.78 <sup>b</sup> (11.00)
	Methanol	0.2±0.44 <sup>a</sup> (1.00)	0.4±0.54 <sup>a</sup> (2.00)	1.2±0.44 <sup>b</sup> (6.00)	1.4±0.54 <sup>b</sup> (7.00)
	Aqueous	6.0±2.23 <sup>b</sup> (30.00)	7.8±4.76 <sup>b</sup> (39.00)	9.8±3.42 <sup>b</sup> (49.00)	10.4±2.07 <sup>b</sup> (52.00)

Mean values of five replicates per trials ±standard deviation. Values in parenthesis denote % mosquitocidal mortality. Different superscript alphabets specify statistical significant difference in mosquitocidal mortality between concentrations at P<0.05 level by one way ANOVA followed by Tukey's test.

population of *Cx. quinquefasciatus* has been reported worldwide and as a result of which plant based products may be considered as alternate biopesticide in Integrated Vector Management Programme (Bowers *et al.*, 1995; Tennyson *et al.*, 2012).

Outcome of the present investigation can be compared with previous reports on the ovicidal activity against *Cx. quinquefasciatus*, where benzene leaf extract of *Ervatamia coronaria* was more effective in exerting ovicidal and larvicidal activity at 300, 250 and 200 ppm against the egg/

Table 2. Probit analysis of ovicidal, larvicidal and pupicidal activity of *N. nucifera* crude leaf extract against *C. quinquefasciatus*

Stages	Solvents	LC <sub>50</sub> (ppm)	95% Confidence Limit		LC <sub>90</sub> (ppm)	95% Confidence Limit	
			LL(ppm)	UL(ppm)		LL(ppm)	UL(ppm)
Ovicidal	Hexane	6.916	6.119	8.711	9.677	8.123	13.504
	Benzene	4.247	4.078	4.420	5.881	5.617	6.213
	Ethyl Acetate	5.732	5.428	6.130	8.168	7.139	8.514
	Methanol	8.640	7.460	11.050	12.774	10.542	17.463
	Aqueous	6.081	5.688	6.628	8.945	8.113	10.203
Larvicidal	Hexane	7.999	7.111	9.892	10.745	9.134	14.299
	Benzene	6.694	6.188	7.465	9.628	8.599	11.290
	Ethyl Acetate	7.968	7.064	9.652	11.537	9.805	14.878
	Methanol	9.933	7.823	18.360	14.223	10.531	29.361
	Aqueous	11.515	8.696	26.653	15.937	11.342	41.005
Pupicidal	Hexane	5.386	4.977	5.964	7.760	6.948	9.168
	Benzene	4.975	4.563	5.521	7.349	6.565	8.711
	Ethyl Acetate	9.059	7.676	12.820	12.291	9.898	18.959
	Methanol	9.988	8.106	16.328	13.716	10.540	24.629
	Aqueous	5.211	4.710	5.953	8.165	7.110	10.133

LC50: Lethal concentration that causes 50% mortality of the exposed larvae; LC90: Lethal concentration that causes 90% mortality of the exposed larvae.

Table 3. Phytochemicals isolated from benzene extract of *N. nucifera*

S. No.	Retention time (RT)(mins)	Peak area %	Name of the compound	Molecular formula	Molecular weight
1	19.12	9.02	Hexadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256
2	20.75	8.74	Hexadecatrienoic acid	C <sub>16</sub> H <sub>26</sub> O	234
			Linoleyl alcohol	C <sub>18</sub> H <sub>34</sub> O	266
3	23.38	3.15	Nornuciferine	C <sub>18</sub> H <sub>19</sub> No <sub>2</sub>	281
4	23.70	29.40	Nuciferine	C <sub>19</sub> H <sub>21</sub> No <sub>2</sub>	295
			Steporphine	C <sub>18</sub> H <sub>17</sub> No <sub>3</sub>	295
			Mecambroline	C <sub>18</sub> H <sub>17</sub> No <sub>3</sub>	295
5	24.36	4.86	Roemerine	C <sub>18</sub> H <sub>17</sub> No <sub>2</sub>	279
6	27.79	7.44	10 Nondecanol	C <sub>19</sub> H <sub>40</sub> O	284
7	29.27	12.00	Oxirane,tetradecyl	C <sub>16</sub> H <sub>32</sub> O	240
			Tetradecanal	C <sub>14</sub> H <sub>28</sub> O	212

egg rafts and larvae of *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*, respectively (Govindarajan *et al.*, 2011). Earlier studies on *N. nucifera* methanol and aqueous leaf extracts indicated higher larvicidal activity against *An. subpictus* and *Cx. quinquefasciatus* (Santhosh kumar *et al.*, 2011; Kamaraj *et al.*, 2011). Ethyl acetate extracts of seed coats of *N. nucifera* had significant larvicide activity (Ray *et al.*, 2014).

*Citrullus vulgaris* benzene leaf extracts yielded 100 per cent mortality at 250 ppm on the eggs of *An. stephensi* after 48h period (Mullai *et al.*, 2008). Reports on *Cardiospermum halicacabum* methanol and benzene leaf extracts revealed complete mortality at 300 ppm against *Cx. quinquefasciatus* eggs (Govindarajan, 2011).

Pupicidal activity against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* was noted in petroleum benzene leaf extract of *Acanthospermum hispidum* (Vivekanandhan *et al.*, 2018). While methanol leaf extract of *Annona reticulata* exhibited effective pupicidal activity of 98.90% at 200 ppm concentration against *Cx. quinquefasciatus* (Selvakumar *et al.*, 2015). Methanol leaf extracts of *Euphorbia hirta* exhibited pupicidal activity against *An. stephensi* was reported by Panneerselvam *et al.* (2013).

GCMS analysis of the benzene extracts of *N. nucifera* revealed the major compounds as neuciferine, steporphine and mecambroline which are aporphine alkaloids which probably have insecticidal properties. Paudel and Panth (2015) reported that the bioactive compounds present in *N. nucifera* are mainly alkaloids and flavonoids. GC-MS of the leaves of *N. nucifera* shows a rich source of a number of alkaloids which exhibit pesticidal properties (Kunitomo *et al.*, 1964; Chen *et al.*, 2013; Do *et al.*, 2013). In the present study it is concluded that the leaf extract of *N. nucifera* has mosquitocidal property against all the stages of *Cx. quinquefasciatus*.

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## Influence of rice crop stage on the distribution of hymenopteran parasitoids of insect pests

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**ABSTRACT:** A total of 43 insect parasitoid species belonging to fourteen families (Aphelinidae, Braconidae, Ceraphronidae, Diapriidae, Encyrtidae, Eulophidae, Eurytomidae, Ichneumonidae, Megaspilidae, Mymaridae, Platygasteridae, Proctotrupidae, Pteromalidae, Trichogrammatidae) has been documented in the rice ecosystem using yellow pan trap. The observations were made at four important stages of rice crop like early tillering, active tillering, booting and panicle development. The parasitoids were also compared with the occurrence of sixteen insect pests that were recorded simultaneously in each stage of the crop. The result revealed that, there is a significant difference in the occurrence of parasitoids according to the stage of the crop and insect host availability. This understanding help in the introduction of specific parasitoids at respective stages for effective biocontrol.

**KEY WORDS:** Stages of rice, parasitoids, yellow pan trap, insect pests

### INTRODUCTION

Rice is an important food crop and India plays a significant role in its production. India has approximately 44 million hectares of land under rice cultivation. But the increasing human population in the country like India have created more demand for food crops. With demand on food crop increase on one hand, the population of insects also are likely to increase due to global warming. This can cause serious loss to the agricultural ecosystem. Global yield losses of these grains due to pests and pathogens are projected to be around 24.6 to 40.9

per cent (Savary *et al.*, 2019). In order to effectively and sustainably control the insect pests, biological control by the use of natural enemies is highly demanded. However good understanding on the diversity of natural enemies in each stage of crop development is lacking. Among the natural enemies, hymenopteran parasitoids are highly significant as it can be used as efficient biocontrol agents due to its host specificity (Dey *et al.*, 1999).

In order to monitor the activity of these parasitoids in rice ecosystem, yellow pan trap proves to be highly efficient (Daniel *et al.*, 2018). This trap

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works on the principle of insect's attractive response towards yellow colour (Kennedy *et al.*, 1961; Hollingsworth *et al.*, 1970). The current study uses this trap to effectively monitor the variation in the occurrence of the parasitoid species at four important stages of rice crop like early tillering, active tillering, booting and panicle development. These stages were also monitored for the prevalence of insect pests so as to understand the relationship of pest and parasitoid populations at specific host plant stages. This understanding will help in better implementation of biocontrol in integrated pest management programs.

## MATERIALS AND METHODS

A paddy field of 400 square meters was taken for the study. The field was located at the wetland, Tamil Nadu Agricultural University, Coimbatore. GPS coordinates of the location is 11.0031° N, 76.9249° E. Inside the field, 16 locations were selected for sampling. Samples were drawn at the four important stages of rice crop like early tillering (2-3 leaf stage), active tillering, booting and panicle development.

Yellow pan traps were used for the collection of parasitoids. The trap pans are bright yellow in colour with a size of 133mm x 195mm and a depth of 48 mm. The traps were filled to 3/4<sup>th</sup> with soap solution mixture. The mixture consists of water, drops of liquid detergent (to break the surface tension) and a pinch of salt (to reduce the rate of evaporation and prevent rotting of trapped insects). At each stage of the crop the traps were placed randomly in 16 places in the field. The traps were placed in between the plants over a flat heap made of clay soil in order to prevent its floating in the field water. After a day, the traps containing the insects were collected in a polythene cover and taken to the laboratory for further washing and preservation. The specimens were preserved in 70% ethanol and refrigerated. Sorting and Identification of the specimens were done under the Leica stereo zoom microscope (Model: M205 C) and also with the help of Stemi (Zeiss) 2000C stereo zoom compound microscope. Taxonomic literature and keys of authors like Narendran (1994), Jonathan

(2006), Rajmohana (2006) and Sureshan (2008) were used for initial identification. In addition, help was also taken by referring to the already identified collection of parasitoids at Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore. The identified parasitoid species were also verified from the list of parasitoids in rice ecosystem published by Daniel and Ramaraju, (2019) and Noyes, (2017). Identified collections are deposited at Insect Biosystematics laboratory, Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore.

Some of the major and minor insect pests of the rice were also recorded during these four stages of the plant. At each stage of the crop, 16 plots were selected and in each plot 15 plants were observed *insitu* for the occurrence of hopper pests and thrips. Sweep net collection was made randomly at sixteen places in the field for remaining pests. Return sweeping were done twice in each place at each stage of the crop.

Shapiro-Wilk normality test was applied to the data using R to check for normality distribution of the data. Since the data was not normally distributed (P value < 0.05), non-parametric Kruskal wallis test was used for analysis. Both the P and H value to the test were noted. These statistical analyses were performed using SPSS.

## RESULTS AND DISCUSSION

A total of 43 hymenopteran parasitoids were observed in the yellow pan trap collection. There belonged to families like Aphelinidae, Braconidae, Ceraphronidae, Diapriidae, Encyrtidae, Eulophidae, Eurytomidae, Ichneumonidae, Megaspilidae, Mymaridae, Platygasteridae, Platygasteroidea, Proctotrupidae, Pteromalidae and Trichogrammatidae.

Significant differences in the occurrence of parasitoids at each stage of rice were observed using the non-parametric Kruskal wallis test in fourteen parasitoid species. They include *Apanteles* sp. (P value = 0.026), *Opius* sp. (P = 0.000), Undetermined diapid (P = 0.001), *Tetrastichus* sp. (P = 0.001), two undetermined eulophid species

( $P = 0.001$ ), *Anagyrus* sp. ( $P = 0.000$ ), *Dicopus* sp. ( $P = 0.000$ ), *Lymanon* sp. ( $P = 0.000$ ), *Mymar* sp. ( $P = 0.028$ ), *Baeus* sp. ( $P = 0.000$ ), undetermined species of platygasteroidea ( $P = 0.006$ ), *Telenomus* sp. ( $P = 0.047$ ) and *Trichogramma* sp. ( $P = 0.002$ ) (Table 1). Similar to the parasitoids, insect pests (except flea beetle) were also found to differ significantly according to the age of the crop (Table 2).

The variation in the occurrence of parasitoids according to the stage of the crop can be compared with the pest infestation in the field. Pests like thrips, yellow stem borer, leaf folder, green leaf hopper, brown plant hopper, white backed plant hopper, white hopper, hispa, green horned caterpillar, skipper, flea beetle, grass hopper, black bug, ear head bug, hairy caterpillar and stink bug were recorded during the four stages of rice plant. Higher occurrence of

rice yellow stem borer *Scirpophaga incertulas*, Walker were found on booting stage followed by panicle development stage during which its egg parasitoids like *Tetrastichus*, *Trichogramma* and *Telenomus* were very minimum. However, the larval parasitoids like Ichneumonids were very active during booting stage. This implies that the parasitoids of stem borers are found to occur according to the developmental stage of the crop and insect host availability. *Anagyrus* sp., an egg parasitoid of plant and leaf hoppers was higher in the early tillering stage keeping the population of the hoppers in control. Other mymarids were found to be prevalent throughout the stages of the rice plant according to the availability of the hopper. Like the parasitoids of yellow stem borer, mymarids (parasitoids of hoppers) were also observed to show preferences according to the stage of the crop in relation to pest occurrence. Larval parasitoids viz.

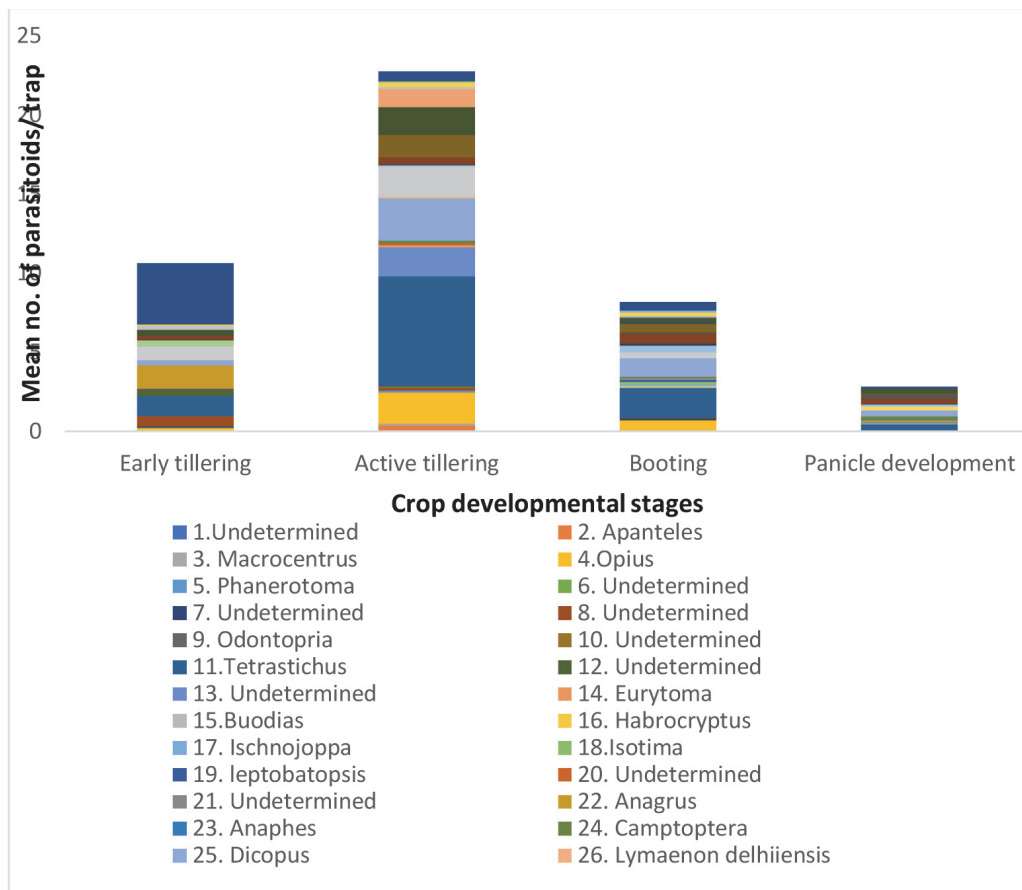


Fig. 1. Hymenopteran parasitoids occurrence in each stage of rice crop

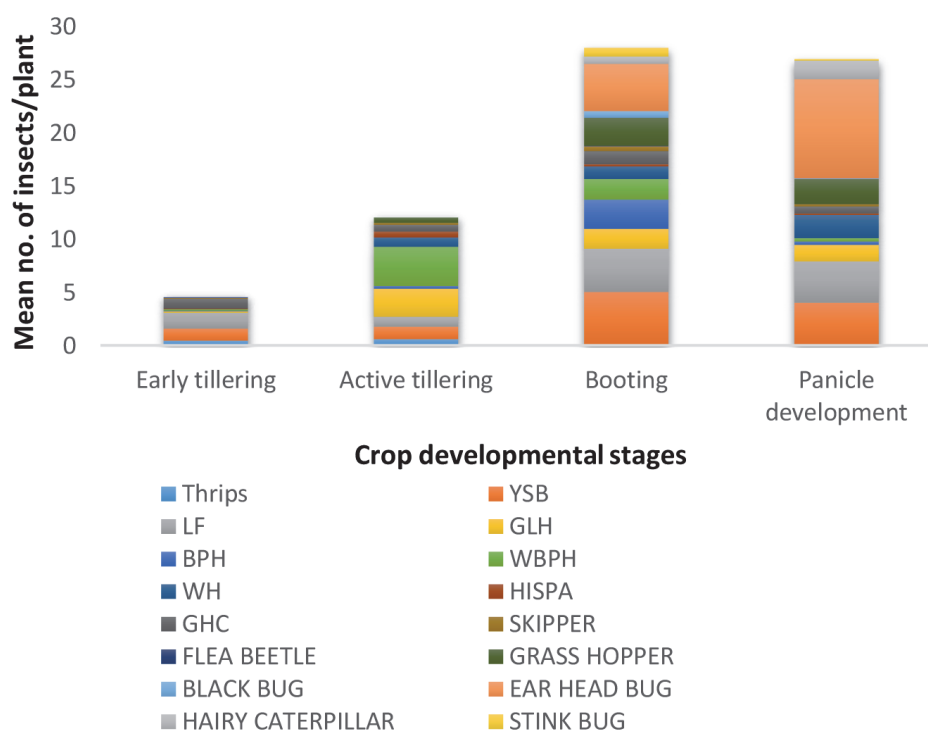


Fig. 2. Distribution of rice insect pests in each stage of rice crop

braconids (*Apanteles* sp.) and ichneumonids were observed to be active in each stages of the rice plant and managed the population of other minor pests like rice horned caterpillar and rice skipper (Devi and Varatharajan, 2013; Hendawy *et al.*, 2016). Certain parasitoid species like *Tetrastichus*, *Trichogramma*, *Duta indica*, *Calliscelio indicus*, *Dicopus* sp. and certain unidentified platygastroidea were found throughout the stages of rice plant. These results imply that the parasitoids are distributed throughout the period of host availability however, their occurrence is also dependent on the stage of the crop development. Their significant difference according to the stage of the crop was also confirmed with the statistical analysis.

Host specific occurrence of the parasitoids were also observed in case of egg parasitoids of rice ear head bug. The *Gryon orestes* was observed only in the rice booting and panicle development stage according to the period of their host occurrence. The egg parasitoid of grasshopper *Duta indica* and

*Calliscelio indicus* were observed throughout the stages of the plant though the grasshoppers were not observed in the early tillering stage of the crop. The presence of *Duta* during the early tillering stage of the crop might be due to the attraction of the parasitoid from the neighbouring field with matured rice crop. Relationship between the insect host and parasitoids was also reported by Daniel *et al.* (2019).

From the results of the present study, it is interesting to observe the fluctuation in the occurrence of parasitoid species according to the developmental stage of the crop irrespective of the host availability. Similar trend was observed among the parasitoids of *Spodoptera frugiperda* in maize (Durocher-Granger *et al.*, 2020). Overall, among the four stages of rice observed, the presence of parasitoids were higher during the active tillering stage followed by early tillering of the crop (Fig. 1). While a greater number of pests occurred in the booting stage followed by panicle



Table 1. Distribution of parasitoids in each stage of the rice crop

No.	Parasitoids	Family	Mean no. of parasitoids/trap				Kruskal wallis test	
			Early tillering stage	Active tillering stage	Booting stage	Panicle stage	H value	P value
1.	Undetermined species	Aphelinidae	0.00	0.00	0.12	0.00	3.000	0.392
2.	<i>Apanteles</i> sp.	Braconidae	0.00	0.71	0.00	0.00	9.289	0.026*
3.	<i>Macrocentrus</i> sp.	Braconidae	0.00	0.24	0.00	0.00	6.097	0.107
4.	<i>Opius</i> sp.	Braconidae	0.35	3.65	1.18	0.00	17.939	0.000*
5.	<i>Phanerotoma</i> sp.	Braconidae	0.00	0.24	0.00	0.00	6.097	0.107
6.	Undetermined species	Braconidae	0.12	0.00	0.00	0.00	3.000	0.392
7.	Undetermined species	Ceraphronidae	0.12	0.00	0.24	0.00	3.787	0.285
8.	Undetermined species	Diapriidae	1.18	0.35	0.12	0.00	16.371	0.001*
9.	<i>Odontopria</i> sp.	Diapriidae	0.12	0.00	0.00	0.00	3.000	0.392
10.	Undetermined species	Encyrtidae	0.00	0.12	0.00	0.00	3.000	0.392
11.	<i>Tetrastichus</i> sp.	Eulophidae	2.35	13.18	3.53	0.82	15.535	0.001*
12.	Undetermined species	Eulophidae	0.82	0.00	0.00	0.00	19.525	0.000*
13.	Undetermined species	Eulophidae	0.00	3.41	0.00	0.00	23.113	0.000*
14.	<i>Eurytoma</i> sp.	Eurytomidae	0.12	0.24	0.00	0.00	2.033	0.566
15.	<i>Buodias</i> sp.	Ichneumonidae	0.00	0.00	0.12	0.12	2.032	0.566
16.	<i>Habrocryptus</i> sp.	Ichneumonidae	0.00	0.00	0.12	0.00	3.000	0.392
17.	<i>Ischnojoppa luteator</i>	Ichneumonidae	0.00	0.00	0.12	0.00	3.000	0.392
18.	<i>Isotima</i> sp.	Ichneumonidae	0.00	0.00	0.35	0.00	6.095	0.107
19.	<i>Leptobatopsis indica</i>	Ichneumonidae	0.00	0.00	0.24	0.00	3.000	0.392
20.	Undetermined species	Ichneumonidae	0.00	0.24	0.00	0.00	3.000	0.392
21.	Undetermined species	Megaspilidae	0.00	0.00	0.24	0.24	3.702	0.296
22.	<i>Anagrus</i> sp.	Mymaridae	2.71	0.00	0.00	0.12	27.072	0.000*
23.	<i>Anaphes</i> sp.	Mymaridae	0.00	0.00	0.00	0.12	3.000	0.392
24.	<i>Camptoptera</i> sp.	Mymaridae	0.00	0.35	0.12	0.35	3.575	0.311
25.	<i>Dicopus</i> sp.	Mymaridae	0.59	5.06	2.24	0.71	20.728	0.000*
26.	<i>Lymaenon delhiensis</i>	Mymaridae	0.00	0.12	0.00	0.00	3.0000	0.392
27.	<i>Lymaenon</i> sp.	Mymaridae	1.65	3.65	0.71	0.00	20.352	0.000*
28.	<i>Mymar pulchellum</i>	Mymaridae	0.00	0.00	0.00	0.47	6.095	0.107
29.	<i>Mymar</i> sp.	Mymaridae	0.00	0.12	0.82	0.24	3.000	0.028*
30.	Undetermined species	Mymaridae	0.71	0.00	0.00	0.00	9.136	0.392
31.	<i>Calliscelio indicus</i>	Platygastridae	0.12	0.12	0.24	0.12	0.641	0.887
32.	<i>Duta indica</i> .	Platygastridae	0.47	0.82	1.18	0.59	6.670	0.083
33.	<i>Gryon orestes</i>	Platygastridae	0.00	0.00	0.12	0.47	9.183	0.027
34.	<i>Baeus</i> sp.	Platygastroidea	0.00	2.71	1.06	0.12	19.758	0.000*
35.	<i>Macroteleia</i> sp.	Platygastroidea	0.00	0.00	0.12	0.00	3.000	0.392
36.	Undetermined species	Platygastroidea	0.71	3.29	0.59	0.59	1.731	0.630
37.	Undetermined species	Platygastroidea	0.00	0.00	0.12	0.00	3.000	0.392
38.	Undetermined species	Platygastroidea	0.00	2.12	0.00	0.00	12.584	0.006*
39.	<i>Telenomus</i> sp.	Platygastroidea	0.47	0.24	0.00	0.00	7.966	0.047*
40.	Undetermined species	Proctotrupidae	0.12	0.59	0.47	0.00	5.677	0.128
41.	Undetermined species	Pteromalidae	0.00	0.00	0.24	0.00	6.097	0.107
42.	<i>Trichogramma</i> sp.	Trichogrammatidae	0.00	0.12	0.00	0.00	3.000	0.392
43.	<i>Trichogramma</i> sp.	Trichogrammatidae	7.29	1.18	1.06	0.24	15.325	0.002*

\*Indicates significant value ( $p < 0.05$ ) of non-parametric Kruskal wallis test.

Table 2. Distribution of rice insect pests according to the stage of crop development

No.	Pests	Mean no. of pests/plant or sweep				Kruskal wallis test	
		Early tillering stage	Active tillering stage	Booting stage	Panicle development stage	Chi square value	P value
1.	Thrips	0.8	1	0	0	8.853	0.031*
2.	Yellow stem borer	1.8	1.9	8.1	6.5	26.140	0.000*
3.	Leaf folder	2.4	1.5	6.5	6.2	18.101	0.000*
4.	Green leaf hopper	0.2	4.2	3	2.5	24.391	0.000*
5.	Brown plant hopper	0.1	0.4	4.4	0.5	25.430	0.000*
6.	White backed plant hopper	0.2	5.9	3.1	0.5	24.618	0.000*
7.	White hopper	0	1.4	1.9	3.5	27.125	0.000*
8.	Hispa	0.1	0.9	0.3	0.2	7.299	0.063*
9.	Green horned caterpillar	1.5	1	2	1.1	10.139	0.017*
10.	Skipper	0.1	0.3	0.7	0.3	5.035	0.017*
11.	Flea beetle	0.1	0	0.1	0	2.032	0.566
12.	Grass hopper	0	0.8	4.2	3.8	37.552	0.000*
13.	Black bug	0	0	1	0.1	12.551	0.006*
14.	Ear head bug	0	0	7.1	14.9	55.954	0.000*
15.	Hairy caterpillar	0	0	1.1	2.8	37.684	0.000*
16.	Stink bug	0	0	1.3	0.2	20.631	0.000*

\*Indicates significant value ( $p < 0.05$ ) of non-parametric Kruskal wallis test.

development stage of the crop (Fig. 2). The occurrence of pests was increased when the population of parasitoids in the field is reduced. So, the conservation of insect natural enemies for effective pest management is a criterion to be taken care in IPM. Moreover, the understanding of the role of parasitoids throughout the stages of crop development is critical to assess timely and effective conservation practises.

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## Revision of the Asian pseudoscorpion genus *Tullgrenius* Chamberlin, 1933 (Pseudoscorpiones: Atemnidae: Miratemninae), a tale of intraspecific variation

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**ABSTRACT:** The Asian pseudoscorpion genus *Tullgrenius* Chamberlin, 1933 is revised. A neotype is designated for *Tullgrenius indicus* Chamberlin, 1933, based on topotype material and a detailed description of its male is provided. Two new synonymies are proposed: *Tullgrenius vachoni* Murthy, 1962 **syn. nov.** and *Tullgrenius orientalis* Sivaraman, 1980 **syn. nov.** = *T. indicus*. Two distinct colour morphs of *T. indicus* are recognized: a brown and black morph. Supplementary descriptions and illustrations for *Tullgrenius afghanicus* Beier, 1959 and *Tullgrenius compactus* Beier, 1951 are detailed with current distribution of all the known *Tullgrenius* spp..

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**KEYWORDS:** Morphology, neotype, synonymies, Western Ghats, India

### INTRODUCTION

The family Atemnidae Kishida, 1929 represents a small family of pseudoscorpions currently having 21 extant and 1 extinct genera, the latter is from a Baltic amber (Beier 1955). Members of the family are bark- as well as litter-dwellers and are grouped into two subfamilies: Atemninae Kishida, 1929 with 15 genera and Miratemninae Beier, 1932 with six genera (Harvey 2013).

The genus *Tullgrenius* Chamberlin, 1933 of Miratemninae, which is restricted to the Oriental region (Harvey 2013), was established by Chamberlin (1933) as a monotypic taxon with the single species, *T. indicus*. Beier (1951) identified the specimen collected by Dawydoffi from Cambodia during the Indochina Expedition (1938–

1939) as *T. compactus*. Later Beier (1959) identified *T. afghanicus* from the collections made by J. Klapperich during the Afghanistan Expedition (1952–1953). In 1962, Murthy described *T. vachoni* from Krusadai Island in the Gulf of Mannar Marine National Park and later, Sivaraman (1980) added *T. orientalis* from Tambaram, Tamil Nadu to the genus.

The unidentified pseudoscorpion specimens in the collections of Murthy and specimens of *T. indicus* collected by Sivaraman (1982), all the specimens are preserved at Loyola College, Chennai, Tamil Nadu were examined. Even though Sivaraman (1980) provided the type locality of his specimens, he never mentioned specific locations. Klausen (2005) collected *Tullgrenius* specimens from south India and identified them as *T. indicus* (Klausen,

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personal communication). Murthy (1983) collected specimens of *T. indicus* from Loyola College campus.

Murthy and Ananthakrishnan (1977) as well as Sivaraman (1980) identified *T. orientalis* based on the length of the pedipalpal segments and trichobothrial positions and ignored the genital features for identification. Chamberlin (1933) and Klausen (2005, 2009) provided good illustrations of male genitalia of *T. indicus*. The inconsistencies in the literature of Sivaraman (1980) and Murthy (1962) indicate that the species classification is not reliable.

The current investigation intends to explain the taxonomy of Indian *Tullgrenius* by comparing the male genital features rather than relying on the variable characters such as length of pedipal segments, rallum of chelicerae, position of trichobothria, which were considered for delineating the species in the past by Indian authors.

## MATERIALS AND METHODS

*Tullgrenius* species were collected from a radius of 30 km, in and around the Nungambakkam City in Tamil Nadu. Perceiving the similarity of these specimens with *T. indicus*, collected *Tullgrenius* specimens from the Eastern Ghats and other regions of Tamil Nadu.

The type specimens of V.A. Murthy collections (VAM colls.) and Sivaraman which were deposited in the museum of Department of Zoology, Loyola College, Chennai are missing and were confirmed with personal observation and communication with S. Sivaraman and D. Sudarsanam. Thus, the revision of topotypes will end the ambiguity in the identity of the species. As the type specimens of *T. indicus*, *T. orientalis* and *T. vachoni* are lost, topotype materials were collected for redescription and the genitalia were examined in detail. All the accessible materials in the genus (113 ♂♂, 87 ♀♀) were examined and also the detailed study of the genitalia is prepared on the basis of the newly collected specimens from India. Moreover, two species, *T. vachoni* and *T. orientalis* are regarded as new synonymies of *T. indicus*. Additional

illustrations and measurements are provided for *T. compactus* and *T. afghanicus*.

The specimens were preserved in 70 per cent ethanol and studied under a Leica M205C, a Zeiss Stemi SV 6 and a Nikon SMZ25 stereomicroscopes and a Nikon ECLIPSE Ni compound microscope. Drawings were made by the aid of a drawing tube. Photographs were taken in a JEOL Model JSM-6390 LV scanning electron microscope available at the Sophisticated Test & Instrumentation Centre (STIC) facility of Cochin University of Science and Technology (CUSAT), Cochin, Kerala, India. All measurements are in millimeters (mm). The specimens are lodged in the Division of Arachnology, Department of Zoology, Sacred Heart College, Thevara, Cochin, Kerala, India (ADSH) 110 ♂♂, 83 ♀♀, Loyola College, Chennai 2 ♂♂, Muséum d'Histoire Naturelle, Geneva (MHNG) 1 ♂, 1 ♀ and Naturhistorisches Museum Wien, Vienna (NHMW) 3 ♀♀. The holotypes are deposited in the MHNG and NHMW. For morphometric analysis, PCA was done using XLSTAT.

Morphological terminology and mensuration follow Chamberlin (1931), Harvey (1992), Judson (2007) and Harvey *et al.* (2012). The following trichobothrial abbreviations were used: *eb* = external basal; *esb* = external sub-basal; *ib* = internal basal; *isb* = internal sub-basal; *ist* = internal sub-terminal; *est* = external sub-terminal; *it* = internal terminal; *et* = external terminal; *t* = terminal; *st* = sub-terminal; *b* = basal; *sb* = sub-basal.

## RESULTS AND DISCUSSION

### Taxonomy

#### Family Atemnidae Kishida, 1929

#### Subfamily Miratemninae Beier, 1932

#### Genus *Tullgrenius* Chamberlin, 1933

(Figs. 1-7)

*Tullgrenius* Chamberlin, 1933: 263; Murthy and Ananthakrishnan, 1977: 133; Sivaraman, 1980: 359; Harvey, 1991: 481.

**Type species** *Tullgrenius indicus* Chamberlin, 1933, by original designation.

### Redefinition and diagnosis

Rallum with four serrated blades. Chelicera with 5 setae, *bs*, *sbs* and *es* subequal in length and terminally or subterminally denticulate. Basal teeth of serrula exterior longer than the rest. Chaetotaxy of chela unique, Fixed chelal finger with 8 trichobothria: *est* almost basal in position and twice as far from *et* as from *esb*; *eb* and *esb* separated by one areolar diameter; *it*, *ist*, *isb* and *ib* clustered at base of finger; movable chelal finger with 4 trichobothria: *sb* and *st* situated diagonally opposite to *b*; *t* situated medially. Tergites with dentate setae; sternites with pointed setae. *est* almost basal in position and twice as far from *et* as from *esb*. *eb* and *esb* apart by one areolar diameter. *sb* and *st* diagonally opposite to *b*. *it*, *ist*, *isb* and *ib* are clustered at the base. Carapacal furrow indistinct. Carapace, tergites and pedipalps granulated. Eye spots distinct. Tarsus IV with a pseudotactile seta, one third of the segment length removed from its base.

**Distribution:** Asia, including Afghanistan, Cambodia, India, and Thailand (Fig. 7).

*Tullgrenius indicus* Chamberlin 1933 (Figs. 1–4)

*Tullgrenius indicus* Chamberlin 1931: 115, figs. 27b and 143, fig. 38n (illustrations of palp and chela); Chamberlin 1933: 264, fig. a (description and illustration of genitalia ♂); Roewer, 1937: 287; Murthy and Ananthakrishnan 1977: 133–134 (distribution of the species); Sivaraman & Murthy 1980: 163–167, fig. 1a–b (description and illustrations of pedipalp and chela ♀); Sivaraman, 1982: 187–194, fig. 3a–e (illustrations of chela of protonymph, deutonymph, tritonymph, male and female); Harvey, 1991: 481; Klausen 2005: 642, fig. 7 (illustration of male genitalia and distribution); Klausen, 2009: figs. 11, 15–16 (surface texture of carapace, illustrations of male and female genitalia).

*Tullgrenius vachoni* Murthy, 1962: 62–65, figs. a–b (description and illustrations of pedipalp and chela ♂); Beier 1974: 1010 (distribution of the species);

Murthy & Ananthakrishnan, 1977: 134 (distribution of the species), **syn. nov.**

*Tullgrenius orientalis* Sivaraman, 1980: 359–362, fig. 7a–b (description and illustrations of pedipalp and chela ♀), **syn. nov.**

### Material examined

#### Neotype (here designated)

**INDIA: Tamil Nadu:** 1 ♂ (ADSH PS0108), Kancheepuram, Guindy [13°00'41"N 80°13'45"E], 18 m a.s.l., 22 January 2019, M.V. Aneesh leg., under bark of *Azadirachta indica*, by hand.

#### Other materials

**INDIA: Andhra Pradesh:** 6 ♂♂, 2 ♀♀ (ADSH PS0110) Vijayawada [16°30'3"N 80°38'17"E], 20 m a.s.l., 10 October 2019; M.V. Aneesh leg., under bark of *Azadirachta indica*, by hand; **Karnataka:** 3 ♂♂, 3 ♀♀ (ADSH PS0111) Shivamoga [13°59'45"N 75°27'55"E], 690 m a.s.l., 31 Jan. 2020; M.V. Aneesh leg., under bark of *Tamarindus indica*, by hand; **Kerala:** 1 ♀ (Loyola College) Kollam, Kulathupuzha, 21 Feb. 1982, 3 ♂♂, 2 ♀♀ (ADSH PS0118) Theni [9°59'20"N 77°27'37"E], 300 m a.s.l., 31 July 2019, M.V. Aneesh leg., under bark of *Tamarindus indica*, by hand; **Tamil Nadu:** 9 ♂♂, 6 ♀♀ (ADSH PS0112) Coimbatore, Karunya nagar [10°56'12"N 76°44'22"E], 460 m a.s.l., 15 October 2019, M.V. Aneesh leg., under bark, 4 ♂♂, 2 ♀♀ (ADSH PS0113) Coimbatore, [11°7'4"N 77°1'47"E], 390 m a.s.l., 15 October 2019, M.V. Aneesh leg., under bark, 9 ♂♂, 5 ♀♀ (ADSH PS0114) Dindigul, [10°8'56"N 78°12'44"E], 300 m a.s.l., 31 July 2019, M.V. Aneesh leg., under bark of *Tamarindus indica*, by hand, 16 ♂♂, 8 ♀♀ (ADSH PS0123) Kancheepuram, Tambaram [12°55'10"N 80°7'24"E], 40 m a.s.l., 10 December 2018, M.V. Aneesh leg., under bark of *Tamarindus indica*, by hand, 16 ♂♂, 15 ♀♀ (ADSH PS0109) same data as for neotype; "22 January 2019", 1 ♂ (Loyola College) Kattupakkam, 26 March 1980, 1 ♂ (Loyola College) Madras, Tambaram, 27 July 1976, S. Sivaraman leg., under bark, 1 ♂ (MHNG)

Madras (now Chennai), Tambaram, 27 July 1976, S. Sivaraman leg., under bark, 1 ♀ (MHNG) Madras (now Chennai), Anamalai hills, Aliyar Dam, 550 m a.s.l., Besuchet C. and Lobl I. leg. 17 November 1932, 1 ♀ (NHMW 25185) Madras (now Chennai), Murthy leg., 5 ♂♂, 4 ♀♀ (ADSH PS0115) Madurai, [10°0'10"N 78°11'3"E], 140 m a.s.l., 30 July 2019 M.V. Aneesh leg., under bark of *Tamarindus indica*, by hand, 2 ♂♂, 4 ♀♀ (ADSH PS0116; Madurai, 10°4'15"N 78°12'51"E, 230 m a.s.l., 30 July 2019 M.V. Aneesh leg., under bark of *Tamarindus indica*, by hand, 3 ♂♂, 4 ♀♀ (ADSH PS0122) Rameswaram, Krusadai Island, [9°14'49"N 79°12'47"E] m a.s.l., 17 April 2019, M.V. Aneesh leg., under bark of tree, by hand, 6 ♂♂, 4 ♀♀ (ADSH PS0118) Tiruvallur, Avadi [13°8'8"N 80°6'10"E], 30 m a.s.l., 12 December 2018, M.V. Aneesh leg., under bark *Tamarindus indica*, by hand, 4 ♂♂, 6 ♀♀ (ADSH PS0119) Tirunelveli [8°54'9"N 77°20'7"E], 170 m a.s.l., 29 July 2019, M.V. Aneesh leg., under bark of *Tamarindus indica*, by hand, 7 ♂♂, 3 ♀♀ (ADSH PS0120) Vinayaganallur, Kancheepuram [12°45'35"N 80°14'40"E], 10 m a.s.l., 12 August 2019, M.V. Aneesh leg., under bark of *Tamarindus indica*, by hand, 2 ♂♂, 1 ♀ (ADSH PS0121) Viluppuram [11°42'4"N 78°56'22"E], 120 m a.s.l., 9 August 2019, M.V. Aneesh leg., under bark of *Tamarindus indica*, by hand, 16 ♂♂, 13 ♀♀ (ADSH PS0117) Yercaud, Kanavaipudur [11°54'44"N 78°11'1"E], 490 m a.s.l., 29 March 2018, 02 April 2018 & 01 May 2018, M.V. Aneesh leg., under bark of *Tamarindus indica*, by hand.

### Differential diagnosis

*Tullgrenius indicus* can be separated from *T. afghanicus* by the stouter nature of chela [chela (with pedicel) 2.47–2.75 (♀) x longer than broad] whereas in *T. afghanicus* it is less stout [2.85 (♀) x longer than broad]. The pedipalpal chela of *T. indicus* has 48–51 (♀) teeth on the fixed finger and 47–53 (♀) teeth on the movable finger, whereas *T. afghanicus* has 39 (♀) on the fixed and 44 (♀) on the movable finger. *T. indicus* can be separated from *T. afghanicus* by its stouter femur + patella

of leg IV [femur + patella 2.06–2.15 x longer than deep] whereas in *T. afghanicus* it is slender [femur + patella 2.37 x longer than deep]. *T. indicus* can be separated by its longer pedipalpal chela [chela (with pedicel) 1.807–1.904 (♀), chela (without pedicel) 1.700–1.792 (♀) than *T. compactus* [chela (with pedicel) 1.129 (♀), chela (without pedicel) 1.036 (♀)].

### Redescription

*Chelicera* (Figs. 2C, 3A to F, 4A, D, E): all five setae of palm well developed; base of palm moderately granulated (Fig. 2C), with exterior and interior condylar lyrifissures and exterior and interior lyrifissures; *ib*, *isb* and *eb* short and terminally dentate. Lamina interior reduced, lamina exterior thin. Fixed finger with four marginal serrate teeth (Fig. 4A). Rallum with four serrate blades, increasing in length from proximal to distal (Figs. 3D to F). Serrula exterior with 20–21 (♂), 17–19 (♀) blades (Figs. 3A, B). Galea long with four terminals and two sub-terminal rami in males, five terminals and one subterminal in females (Figs. 3C, 4D, E).

*Pedipalps* (Fig. 2A, B, D): trochanter and femur granulated, except ventral surfaces; small setae terminally dentate on entire pedipalp, long setae acuminate. Patella heavily granulated retrolaterally, remainder finely granulated. Chelal hand heavily granulated retrolaterally, remainder finely granulated. Dorsal tubercle of trochanter well developed (Fig. 2D); trochanter 1.27–1.37 (♂), 1.18–1.56 (♀) x longer than broad. Femur 1.86–1.96 (♂), 1.68–1.90 (♀) x longer than broad. Patella 1.68–1.84 (♂), 1.67–1.78 (♀) x longer than broad. Chela with pedicel 2.63–2.80 (♂), 2.47–2.75 (♀) x longer than broad; fixed finger with 45–48 (♂), 48–51 (♀) teeth, movable finger with 51–56 (♂), 47–53 (♀) teeth. Fixed chelal finger with 8 trichobothria: *est* almost basal in position and twice as far from *et* as from *esb*; *eb* and *esb* separated by one areolar diameter; *it*, *ist*, *isb* and *ib* clustered at base of finger; movable chelal finger with 4 trichobothria: *sb* and *st* situated diagonally opposite to *b*; *t* situated medially (Figs. 2A, B).



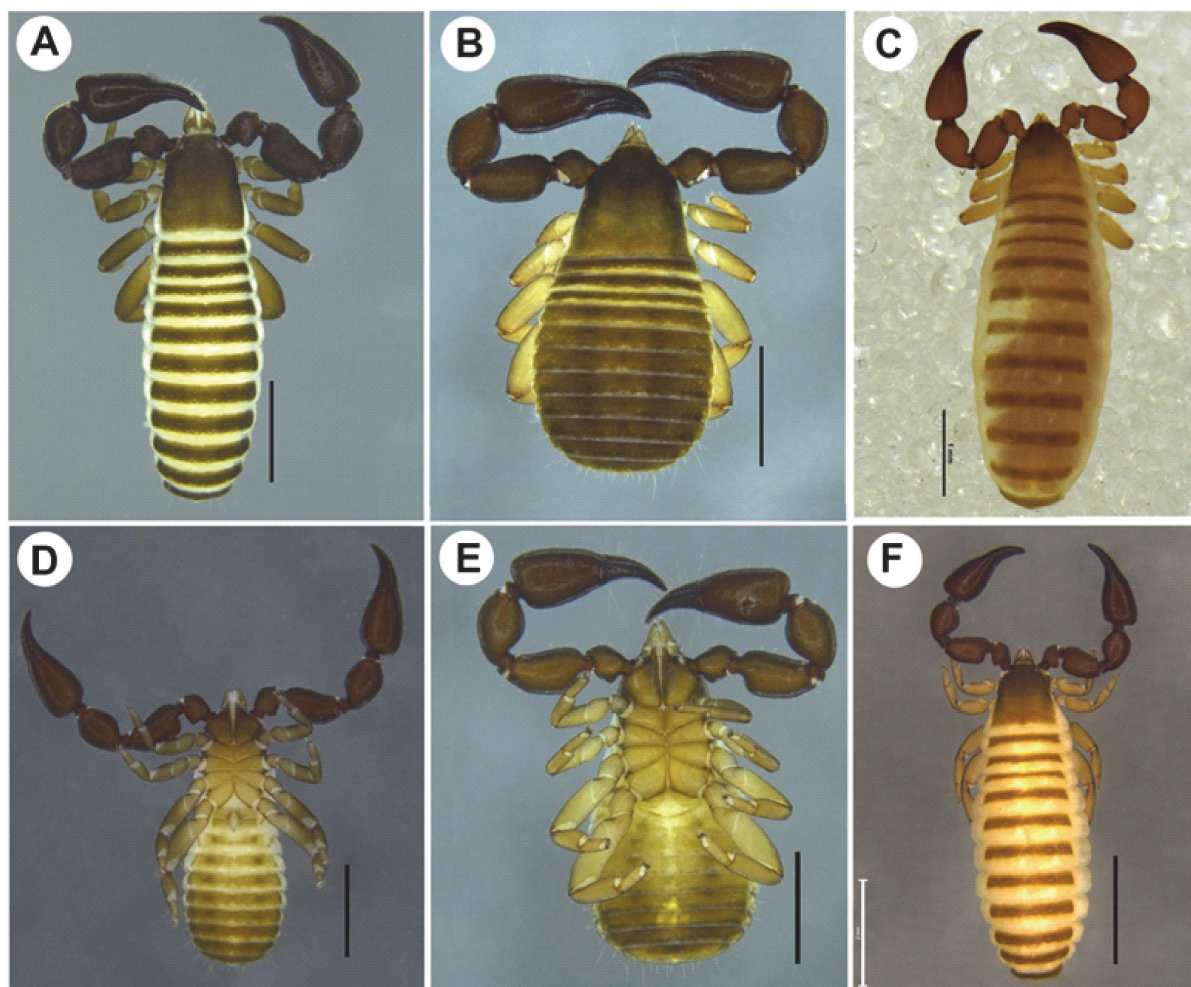


Fig. 1 *Tullgrenius indicus* - A. male Neotype, dorsal view; B. female dorsal view; C. female dorsal view (NHMW 25185); D. male Neotype, ventral view; E. female ventral view; F. female dorsal view (ADSH PS0112). Scale bars: A–D, 1mm.

**Carapace** (Fig. 3H to I): 0.94–1.18 (♂), 1.0–1.05 (♀) x longer than broad, granulated with two distinct eye spots, with two indistinct furrows (Fig. 3I), with ca. 42 (♂), 38 (♀) setae, including 4 at anterior margin and 9 near posterior margin. Vestitural setae terminally dentate (Fig. 3H).

**Coxal region:** maxillary lyrifissures situated medially. Coxal chaetotaxy: ♂, 7: 8: 4: 5: 9, ♀, 5: 7: 5: 6: 9.

**Legs** (Figs. 4B, C): light brown, granulated anterolaterally, with long, acuminate setae and small, terminally dentate setae, articulation between femur

and patella oblique of Leg III and IV. Leg I (Fig. 4C): trochanter 0.97–0.98 (♂), 0.82–0.87 (♀), femur 0.89–0.97 (♂), 0.88–0.90 (♀), patella 1.67–1.80 (♂), 1.71–1.82 (♀), tibia 2.65–2.48 (♂), 2.55–2.63 (♀), tarsus 2.66–2.95 (♂), 2.71–2.81 (♀) x longer than broad. Leg IV (Fig. 4D): femoral lyrifissure present; trochanter 1.51–1.55 (♂), 1.53 (♀), femur+patella 2.12–2.20 (♂), 2.06–2.15 (♀), tibia 2.93–3.11 (♂), 3.02–3.16 (♀), tarsus 2.70–2.92 (♂), 2.62–2.95 (♀) x longer than broad; tactile seta situated one third of the tarsus from its base, TS=0.32.

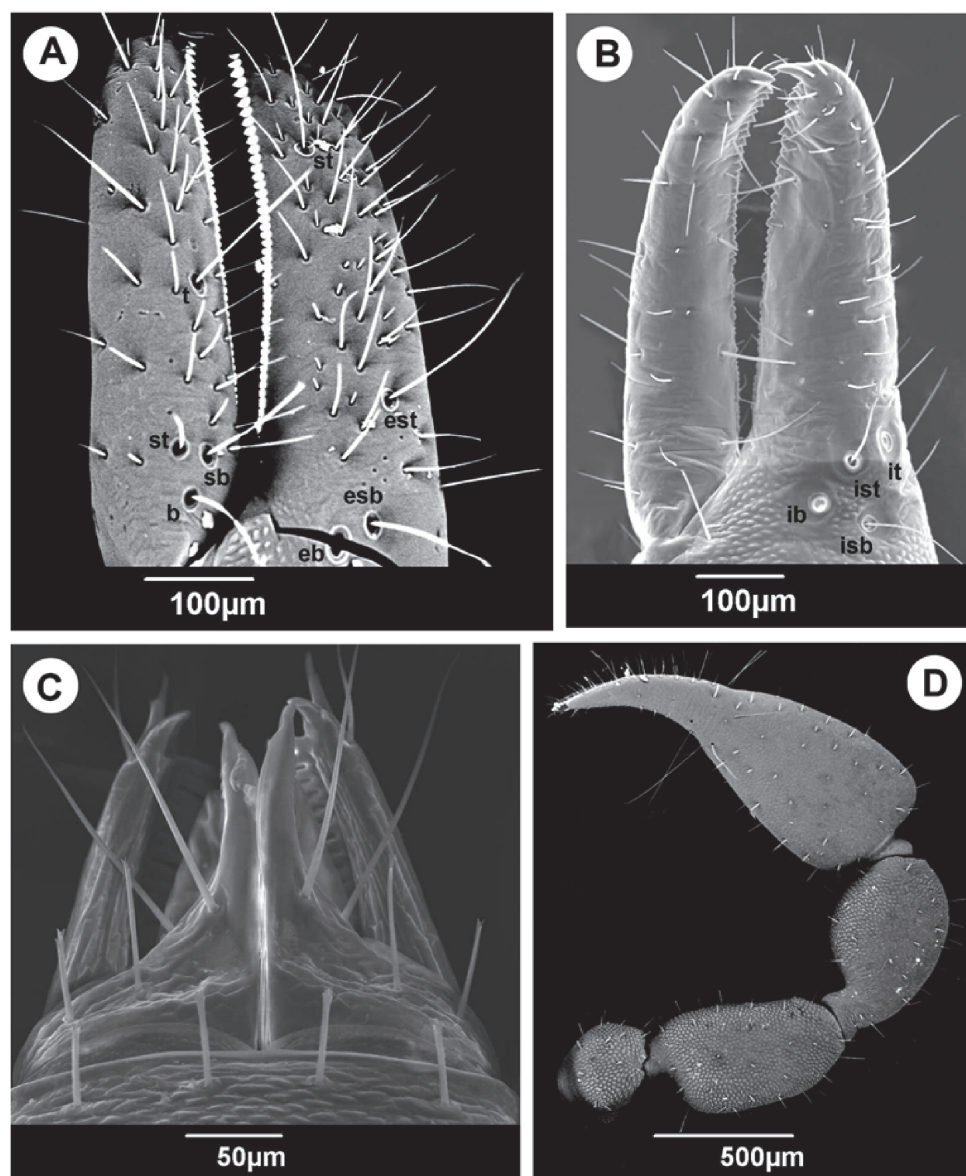


Fig. 2 *Tullgrenius indicus* male. A. Left chela; B. Right chela; C. Chelicerae; D. Right Pedipalp



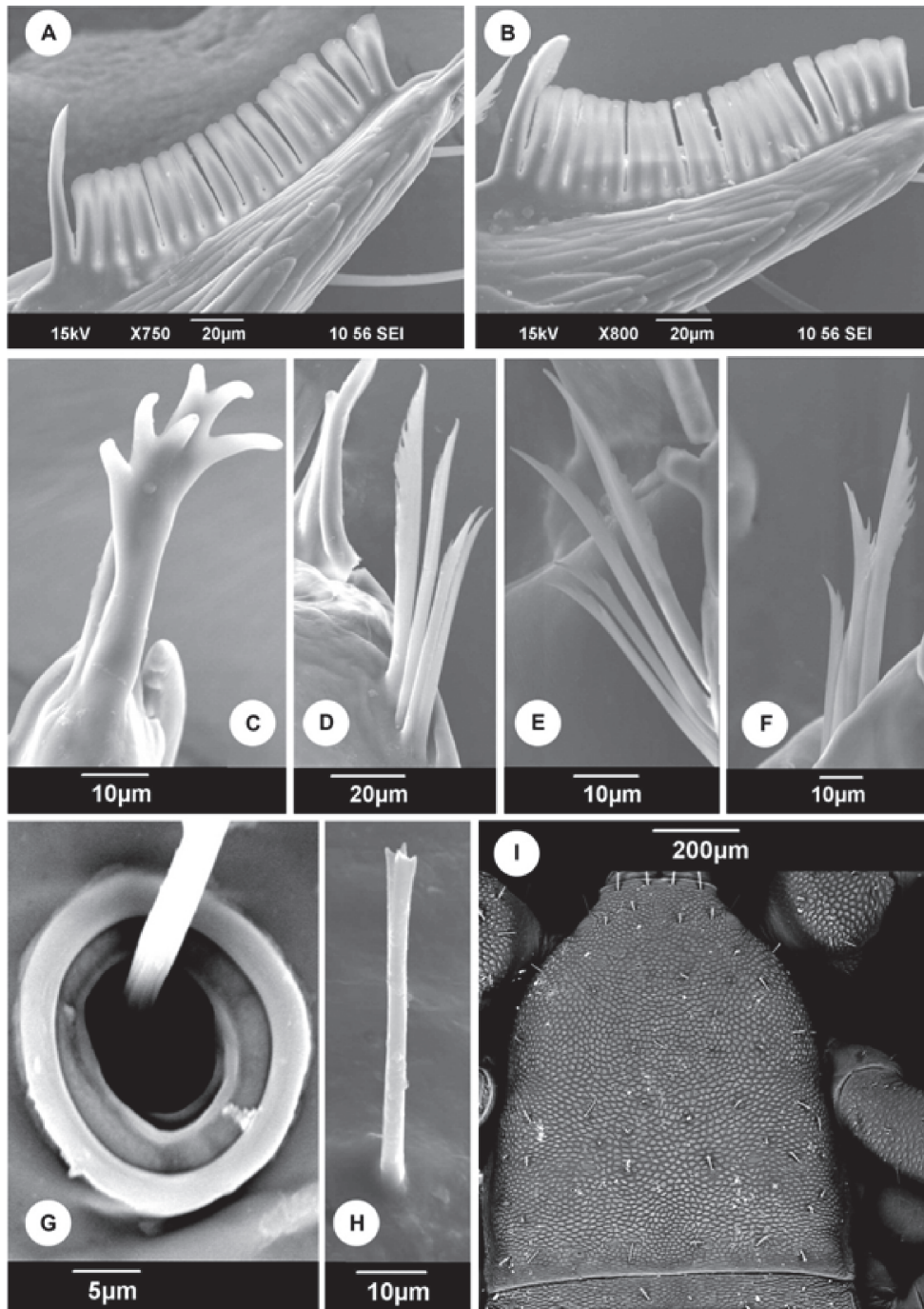


Fig. 3 *Tullgrenius indicus* female. A. Serrula exterior of female; B. Serrula exterior; C. Galea; D to F - Rallum of left chelicera; G. Arolium of *ist*; H. Terminally dentate setae on carapace; I. Carapace

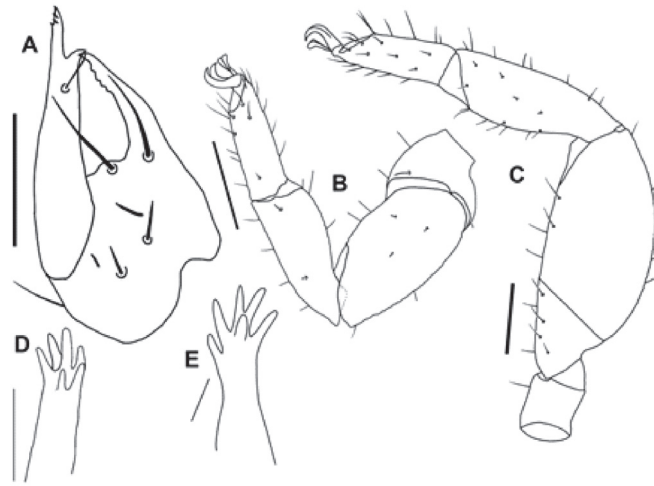


Fig. 4 *Tullgrenius indicus* male (A–E). A. Rallum; B. Left chelicera; C. Left leg I, lateral; D. Left leg IV, lateral; E. Left galea; F. Left galea of female. Scale bars: A–F, 0.2mm

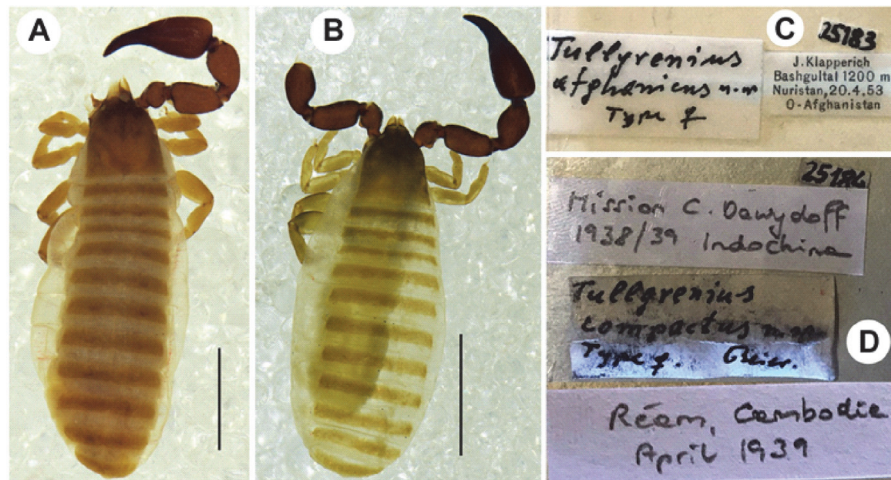


Fig. 5 A - *Tullgrenius compactus* female holotype; B - *Tullgrenius afghanicus* female holotype; C - Original label of *T. afghanicus*; D - Original label of *T. compactus*. Scale bars: A–B 1 mm

*Opisthosoma* (Figs. 1A to F): tergites granulated, with two thick brown patches, one on either side of tergites II and IV to X, which are said to be muscular insertions (Judson, pers. comm.) (Fig. 1A, B, E, F). Tergal chaetotaxy: ♂, 7–9: 8: 10: 10(2): 10(2): 8–11(2): 8–10(2): 8–9(2): 8(2): 9–11 (including 4 tactile setae): 8–11 (including 4 tactile setae): 2, ♀,

10: 10: 12: 10–12(2): 12(2): 12(2): 12–13(2): 12(2): 12(2): 13–15 (including 4 tactile setae): 12–13 (including 2 tactile setae): 2; small setae terminally dentate and tactile setae acute. Sternites IV to X with thick brown patches, one on either side (Figs. 1C, D). Sternal chaetotaxy: ♂, 7: 7: 8: 10: 9: 11: 10: 11: 11 (including 2 tactile setae): 10 (including 4

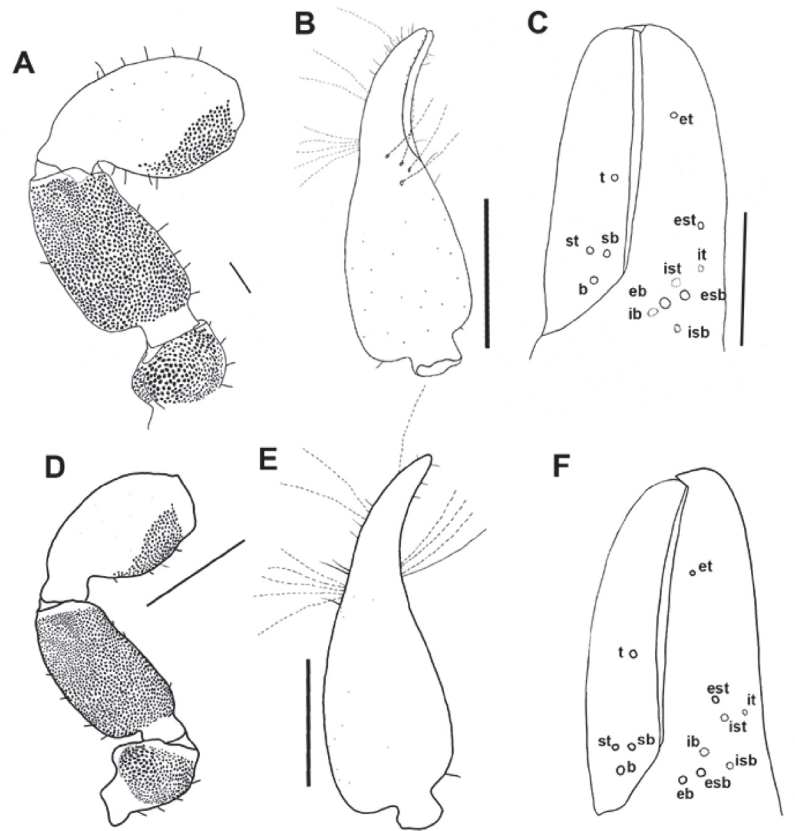


Fig. 6 *Tullgrenius compactus* (A–C). *Tullgrenius afghanicus* (D–F). A - Left pedipalp; B - Left chela; C - Left chela without hand; D - Left pedipalp; E - Left chela; F - Left chela without hand. Scale bars: A, F 0.1mm, C 0.2mm, B, D–E 0.5mm

Table 1. Morphometric data revealing morphological variations among the three *Tullgrenius* species

Species	Patella	Femur	Chela with pedicel	Serrula exterior	Femur ratio	Patella ratio
<i>T. indicus</i>	0.958	0.936	2.47	17	1.68	1.7
<i>T. indicus</i>	0.972	0.956	2.75	19	1.96	1.78
<i>T. indicus</i>	0.921	0.907	2.6	18	1.72	1.78
<i>T. compactus</i>	0.596	0.566	2.6	17	1.9	1.8
<i>T. afghanicus</i>	0.772	0.796	2.85	17	2.2	1.9
<i>T. indicus</i>	0.962	0.946	2.68	19	1.76	1.7
<i>T. indicus</i>	0.942	0.921	2.55	19	1.82	1.76
<i>T. indicus</i>	0.953	0.938	2.67	19	1.8	1.78
<i>T. indicus</i>	0.946	0.923	2.65	17	1.7	1.74
<i>T. indicus</i>	0.956	0.929	2.7	19	1.76	1.67
<i>T. indicus</i>	0.966	0.949	2.5	19	1.76	1.7
<i>T. indicus</i>	0.91	0.902	2.7	17	1.74	1.74
<i>T. indicus</i>	0.917	0.904	2.65	19	1.62	1.7
<i>T. indicus</i>	0.938	0.919	2.54	19	1.6	1.7

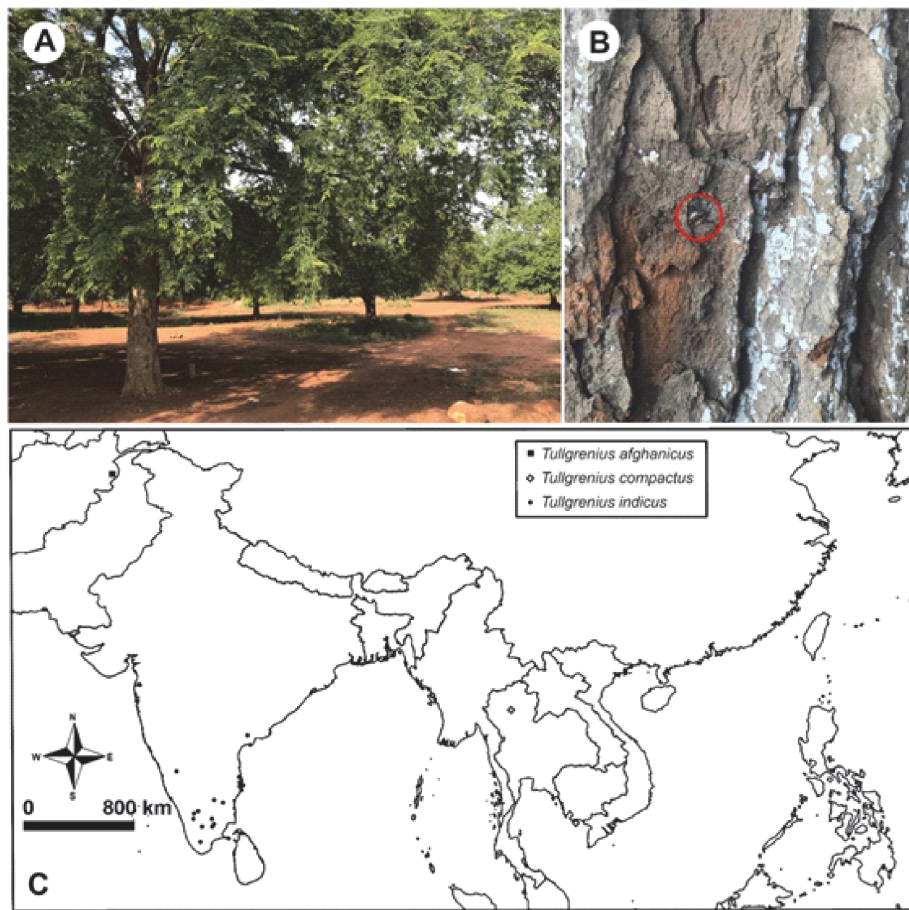


Fig. 7 A, B - Habitat of *Tullgrenius indicus*; C. Geographic distribution of known *Tullgrenius* spp.

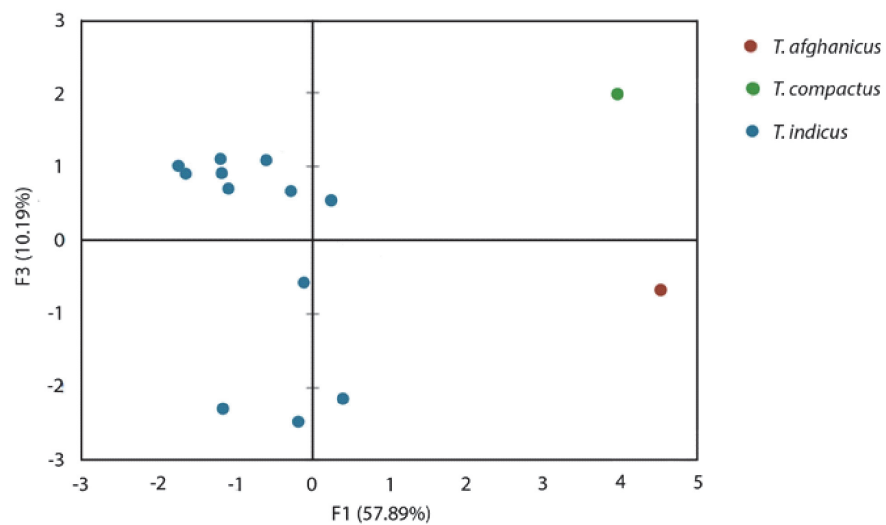


Fig. 8 Principal Component Analysis of morphological features obtained from the three known *Tullgrenius* species



tactile setae): 2, ♀, 6: 8: 10(2): 10(2): 12(2): 9–12(2): 11–13(2): 10–13(2): 13–14 (including 4 tactile setae): 13–14 (including 4 tactile setae): 2; all setae acute.

**Measurements.** *Males*: neotype followed by other males in parentheses: body length 3.5 (3.011–3.889). Carapace 0.988/1.030 (1.072–1.167/0.951–1.119). Pedipalps: trochanter 0.454/0.331 (0.408–0.598/0.380–0.439), femur 0.828/0.445 (0.802–0.921/0.437–0.511), patella 0.829/0.492 (0.789–0.928/0.446–0.532), chela (with pedicel) 1.599/0.576 (1.440–1.767/0.530–0.685), chela (without pedicel) 1.524/0.576 (1.359–1.662/0.530–0.685), hand (with pedicel) 0.855 (0.833–1.053), hand (without pedicel) 0.764 (0.751–0.939), movable finger 0.705 (0.625–0.816). Leg I: trochanter (0.182–0.210/0.187–0.213), femur 0.190/0.237 (0.256–0.278/0.262–0.311), patella 0.418/0.245 (0.474–0.502/0.263–0.30), tibia 0.419/0.156 (0.434–0.517/0.175–0.195), tarsus 0.345/0.106 (0.314–0.405/0.114–0.118). Leg IV: trochanter (0.313–0.358/0.201–0.236), femur+patella 0.818/0.379 (0.873–1.061/0.410–0.481), tibia 0.633/0.226 (0.682–0.832/0.232–0.264-), tarsus 0.424/0.145 (0.424–0.463/0.145–0.171).

*Female*: body length 3.05–4.38. Carapace 1.00–1.07/0.996–1.066. Pedipalps: trochanter 0.418–0.432/0.444–0.454, femur 0.907–0.936/0.531–0.552, patella 0.958–0.972/0.563–0.574, chela (with pedicel) 1.807–1.904/0.695–0.728, chela (without pedicel) 1.700–1.792/0.695–0.728, hand 0.922–1.102, movable finger. 0.797–0.896. Leg I: trochanter 0.150–0.172/0.181–0.196, femur 0.212–0.218/0.240–0.249, patella 0.410–0.416/0.225–0.242, tibia 0.403–0.417/0.158, tarsus 0.318/0.113–0.117. Leg IV: trochanter 0.323–0.338/0.211–0.220, femur + patella 0.797–0.836/0.386–0.388, tibia 0.648–0.672/0.212–0.214, tarsus 0.388–0.396/0.134–0.148.

## Remarks

Chamberlin (1933) doubted about a missing trichobothria, *t* on the movable finger of *T. indicus*. Later, Beier (1951) corrected this and suggested that the missing of trichobothria could not be considered as the characteristics of *Tullgrenius*

species. Murthy (1962) described *T. vachoni* based on a single male holotype. Even though he mentioned one female allotype, he did not describe it. Without providing the details of males other than holotype, Murthy (1962) presented a range for pedipalpal measurements of *T. vachoni* in the description, which is confusing as the measurements of paratypes are not mentioned. He separated *T. vachoni* from *T. indicus*, based on the length of the patella and femur of pedipalp. In the present study, it was confirmed that the difference in the length of the patella and femur of pedipalp could not be considered as a significant feature to separate *Tullgrenius* species. Beier (1974) suggested that *T. vachoni* might be a junior synonym of *T. indicus* and is confirmed in the present study by examining the male genitalia of *T. vachoni*—medial diverticula reduced, two lateral lobes, ventral diverticulum with hood-like appearance (Klausen, 2005: Fig. 7). Sivaraman (1980) separated *T. orientalis* from *T. indicus* by comparing the length of the femur and patella of pedipalp and the nature of the flagellum. All the specimens collected in the present study had the femur longer than patella or of equal in length. From the morphological, morphometric and genital characters, it is confirmed that *T. orientalis* is a junior synonym of *T. indicus*. From the present study, it is clear that the chaetotaxy of the movable finger is complete (Fig. 2A) and the position of the trichobothria is considered to the generic level. The nature of the galea in both sexes are found stable (Figs. 2C, 4E, F). Serrula exterior of all the observed specimens showed variations (17–20) and is not considered significant to delineate species. Murthy (1962) and Sivaraman (1980) observed a second and a third flagella serrated. In the present study, it is confirmed as an intraspecific variation (Figs. 3D to F, 4A). The black coloured form (Figs. 1A to D) is considered as a colour morph of the brown form (Figs. 1E, F).

## Note

The specimen of *T. orientalis* deposited in MHNG is designated as paratype, dated 27 July 1976, collected from Tambaram, Madras, is probably not a paratype.

***Tullgrenius compactus* Beier, 1951** (Figs. 5A, C, 6A, B)

**Type material. Holotype.** CAMBODIA: 1 ♀ (NHMW 25184), Riem [10°30'12"N 103°37'24"E], April 1939, C. Dawydoff leg., examined.

#### Differential diagnosis

*T. compactus* can be separated from other species of the genus by its longer pedipalpal chela [chela (with pedicel) 1.480 (♀), chela (without pedicel) 1.381 (♀)] than *T. afghanicus* [chela (with pedicel) 1.129 (♀), chela (without pedicel) 1.036 (♀)]. The pedipalpal chela of *T. compactus* has 44 (♀) teeth on the fixed finger and 47 (♀) teeth on the movable finger [whereas, it is 39 (♀) teeth on the fixed finger and 44 (♀) teeth on the movable finger of *T. afghanicus*].

#### Redescription

**Chelicera:** all five setae well developed; base of palm fairly granulated, *ib*, *isb*, *eb* short and terminally dentate. Lamina interior reduced, exterior thin. Fixed finger with four marginal serrations. Serrula exterior with 17 (♀) blades.

**Pedipalps** (Fig. 6A to C): reddish-brown; trochanter and femur granulated, except ventral; patella granulated retrolaterally, rest finely granulated. Chelal hand heavily granulated retrolaterally, remainder finely granulated. Dorsal tubercle of trochanter well developed (Fig. 6A); trochanter 1.47 x longer than broad. Femur 1.86 x longer than broad. Patella 1.79 x longer than broad. Chela with pedicel 2.61 x longer than broad; fixed finger with 44 teeth, movable finger with 47 teeth. *eb* and *esb* apart by one areolar diameter. *sb* diagonally opposite to *st. it*, *ist*, *isb* & *ib* are clustered at the base (Fig. 6C). Small setae terminally dentate, long setae acuminate.

**Carapace:** 0.93 x longer than broad, granulated, with two distinct eye spots, with two indistinct furrows, with ca. 38 setae, including 4 at anterior margin and 8 near to posterior margin. Vestitural setae terminally dentate.

**Legs:** brownish, granulated antero-laterally, with acuminate long setae and terminally dentate small setae, articulation between femur and patella oblique of Leg III and IV. Leg I: femur 0.760, patella 1.89, tibia 2.60, tarsus 2.80 x longer than broad. Leg IV: femur+patella 2.45, tibia 3.08, tarsus 2.69 x longer than broad; tactile seta situated one third of the tarsus from its base.

**Measurements. Female:** body length 3.737. Carapace 0.862/0.921. Pedipalps: trochanter 0.367/0.250, femur 0.566/0.303, patella 0.596/0.332, chela (with pedicel) 1.129/0.431, chela (without pedicel) 1.036/0.431, hand 0.560, movable finger 0.517. Leg I: femur 0.111/0.146, patella 0.321/0.169, tibia 0.305/0.117, tarsus 0.227/0.081. Leg IV: femur+patella 0.546/0.222, tibia 0.456/0.148, tarsus 0.267/0.099.

***Tullgrenius afghanicus* Beier, 1959** (Figs. 5B, D, 6C, D)

**Type material. Holotype.** AFGHANISTAN: Nuristan Province: 1 ♀ (NHMW 25183), Bashgultal, [34°56'56"N 70°57'34"E], 1200 m a.s.l., 20 April 1953, J. Klapperich leg., examined.

#### Differential diagnosis

*T. afghanicus* can be separated from other species of the genus by its shorter pedipalpal chela [chela (with pedicel) 1.129 (♀), chela (without pedicel) 1.036 (♀)] than *T. compactus* [chela (with pedicel) 1.480 (♀), chela (without pedicel) 1.381 (♀)]. The pedipalpal chela of *T. afghanicus* has 39 (♀) teeth on the fixed finger and 44 (♀) teeth on the movable finger [whereas, it is 44 (♀) teeth on the fixed finger and 47 (♀) teeth on the movable finger in *T. compactus*].

#### Redescription

**Chelicera:** all five setae well developed; base of palm fairly granulated, *ib*, *isb*, *eb* short and terminally dentate. Lamina interior reduced, exterior thin. Fixed finger with four marginal serrations. Serrula exterior with 17 blades.

**Pedipalps** (Figs. 6D to F): Reddish-brown; trochanter and femur granulated, except ventral;



patella granulated retrolaterally, rest finely granulated. Chelal hand heavily granulated retrolaterally, remainder finely granulated. Dorsal tubercle of trochanter well developed (Fig. 6D); trochanter 1.38 x longer than broad. Femur 2.04 x longer than broad. Patella 1.89 x longer than broad. Chela with pedicel 2.85 x longer than broad; fixed finger with 39 teeth, movable finger with 44 teeth. *eb* and *esb* apart by one areolar diameter. *sb* diagonally opposite to *st*. *it*, *ist*, *isb* & *ib* are clustered at the base (Fig. 6F). Small setae terminally dentate.

*Carapace*: 1.04 x longer than broad, granulated with two distinct eye spots, with two indistinct furrows, with ca. 31 setae, including 4 at anterior margin and 8 near to posterior margin. Vestitural setae terminally dentate.

*Legs*: brownish, granulated antero-laterally, with acuminate long setae and terminally dentate small setae, articulation between femur and patella oblique of Leg III and IV. Leg I: femur 0.949, patella 1.52, tibia 2.81, tarsus 2.70 x longer than broad. Leg IV: femur+patella 2.37, tibia 3.25, tarsus 2.82 x longer than broad; tactile seta situated one third of the tarsus from its base.

*Measurements: Female*: body length 4.690 Carapace 1.062/1.014. Pedipalps: trochanter 0.443/0.319, femur 0.772/0.378, patella 0.796/0.421, chela (with pedicel) 1.480/0.518, chela (without pedicel) 1.381/0.518, hand 0.739, movable finger 0.612. Leg I: femur 0.208/0.219, patella 0.321/0.202, tibia 0.397/0.141, tarsus 0.317/0.117. Leg IV: femur+patella 0.721/0.304, tibia 0.570/0.175, tarsus 0.399/0.141.

## Note

Bashgultal — now referred to as Bashgul Valley/Landai Sin Valley situated near to Pakistan border.

*T. compactus* is known from its type locality and Thailand (Schawaller, 1994) while *T. afghanicus* is known only from its type locality. Both species are separated from each other by differences in the measurements of femur and patella of pedipalp and in the number of teeth on the movable and fixed fingers. From the present study, it is clear that collection from the type localities and further study

on male genitalia is required to get more clarity on the characteristics for delineating species.

## Key to species of the genus *Tullgrenius*

1. Chela with pedicel longer than 1.6mm and less than 2.8 times as long as wide..... *T. indicus*  
Chela with pedicel less than 1.6mm.....2
2. Chela stouter and less than 1.2mm .....*T. afghanicus*  
Chela with pedicel between 1.2mm and 1.6mm ..... *T. compactus*

## Morphometric analysis

Morphometric data revealed morphological variations among the three *Tullgrenius* species and also concluded that the femur and patella length shows high level of correlation and cannot be considered for delineating species. The principal component analysis (Factors 1 and 3 jointly) explained 68.08% of the total variation among species, the first explaining 57.89 per cent and the second explaining 10.19% (Fig. 8). The variables most correlated to Factor 1 were Chela with pedicel, Femur ratio and Patella ratio (Table 1).

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## Impact of seasonal adult emergence period on reproductive performance of tasar silkmoth *Antheraea mylitta* Drury (Lepidoptera: Saturniidae)

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**ABSTRACT:** Influence of adult emergence period and prevailing weather parameters on reproductive biology of tasar silkmoth *Antheraea mylitta* was studied under grainage (indoor) condition. There was a significant difference of fecundity was observed on different days of adult emergence. Maximum fecundity was observed on 10<sup>th</sup> day (219 eggs/female) during first grainage (diapause cocoons) while same has been observed on 13<sup>th</sup> day (224.30 eggs/female) of emergence during second grainage (non-diapause cocoons). With respect to hatching percentage, during first grainage maximum hatching was observed on 1<sup>st</sup>, 6<sup>th</sup>, 7<sup>th</sup> and 12<sup>th</sup> days of emergence (93.32, 90.14, 90.96 and 90.18 % respectively). In the case of second grainage maximum hatching was on 12<sup>th</sup> and 2<sup>nd</sup> day of emergence. Data on per cent egg retention during first grainage ranged between 6.46 to 29.25 % and it was between 6.49 to 14.39 % during second grainage. Retained eggs were unfertile and could observe less than 2 % of hatching across all the days of emergence in first and second grainage together. Despite of significant difference in the reproductive parameters it was not clear about which phase or days of seasonal emergence period yields better layings. Better reproductive performance was scattered randomly across the days of emergence and it also indicates that adult emergence period don't have any influence over reproductive biology of *A. mylitta*. Prevailing temperature and relative humidity during emergence period found to have no major influence over fecundity, hatching percentage and egg retention. © 2021 Association for Advancement of Entomology

**KEYWORDS:** Fecundity, hatching, egg retention, relative humidity

### INTRODUCTION

Indian tasar silk moth *Antheraea mylitta* Drury (Lepidoptera: Saturniidae), is an economically important wild silk moth species distributed across India. Despite high potential of country's tropical tasar industry scarcity of good quality eggs for commercial rearing is one of the major reasons for the decline in Tasar silk production. In tasar

sericulture, pre-seed and seed crops are affected by adverse climatic conditions; diseases and erratic emergence in seed cocoon as a result commercial crops are not getting adequate timely supply of quality seed. The farmers are unable to utilize their full potential of natural plantation for rearing during the favourable commercial crops. Hence, there is a wide gap between the demand and supply of disease free layings (DFLs).

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Most insects survive periods of environmental stress by entering a state of diapause. The Indian tropical tasar silkworm, *A. mylitta* completes two to three generations in a year (Sinha and Chaudhuri, 1992), bivoltine and trivoltine broods undergoes pupal diapause for a period of about six to seven months to overcome unfavourable environmental conditions (Dash and Nayak, 1988, Kapila *et al.*, 1991, Sinha and Chaudhuri, 1992). Pupal diapause in this species normally terminates at the end of May and eclosion begins in June with the advent of rain (Sinha and Chaudhuri 1992). This is known as optimal seasonal emergence. However, in the diapausing brood a portion of the pupae hatch 1-2 months early, emerging in a presumably unfavourable climate before the rainy season (Kapila *et al.*, 1991). The physiological/hormonal basis of this erratic eclosion remains unclear, although endocrine regulation of pupal diapause in other insects has been well documented (Browning, 1981; Denlinger 1985). Daily patterns of insect behaviour (e.g., locomotion, feeding, emergence, mating, oviposition, and hatching) are governed by daily cycles of temperature, humidity, and light intensity as well as by physiological events (Beck 1983, Ashby and Singh 1990). Considering the fact there is a general recommendation is that during oviposition less than 30°C temperature and 70-80% relative humidity is required to be maintained to prevent egg desiccation and poor hatching (Prasad *et al.*, 2000).

Reproductive biology is one of the important factors as per as productive insects are concerned. Generally, it is governed by various factors and they can be broadly categorized into internal (biological) and external (non-biological) factors. The role of temperature and relative humidity on oviposition and incubation of eggs were reported earlier by several workers. However, information on the reproductive performance of *A. mylitta* on different emergence days and influence of prevailing temperature and relative humidity is scanty, with this background during the present study efforts were made to know the impact of adult emergence period over reproductive performance of tasar silkworm along with influence of temperature and relative humidity prevalent during the adult emergence.

## MATERIALS AND METHODS

Study was carried out at Pilot Project Centre, Dept. of Sericulture, Govindpur, Dhanbad, Jharkhand in the month of July and September 2019, covering two grainage periods. Generally in bivoltine tasar silkworm, first grainage (diapause brood) will be performed in June/July months and second grainage (Non diapause brood) will be performed in Aug/Sept months. During the study, adult emergence was monitored regularly during both grainage periods and when the emergence was started, randomly 15 moths were selected and allowed them for mating, out of them 10 females were used for the study. After the mating period selected moths were decoupled and kept for oviposition for 3 days and it has been continued till the emergence period over. In the course of the experiment various reproductive parameters *viz.*, Fecundity, Hatching percentage, Egg retention and hatching percentage in retained eggs were recorded daily for two grainage periods.

**Fecundity:** After the oviposition period of 3 days, fecundity was recorded from individual 10 mother moths (Pebrine free) following the method of Sinha (1998).

**Hatching percentage:** Hatching percentage was calculated by using the formula:

$$\frac{\text{No. of larvae hatched}}{\text{Fecundity}} \times 100$$

Only larvae hatched during first 3 days were considered to calculate hatching %. Similarly hatching % was also calculated for the retained eggs.

**Egg retention in moths:** To analyse the extent of egg retention after the egg laying period, same 10 coupled mother moths (Pebrine free) were dissected individually and number of eggs retained in ovary were recorded and they have been collected and kept for hatching to know their fertility status. Per cent egg retention was calculate by the following formula

$$\frac{\text{No. of retained eggs}}{\text{Fecundity (No of eggs laid)}} \times 100$$



During the present experiment, possible influence of temperature and relative humidity on reproductive performance of *A. mylitta* was also studied. Daily temperature and relative humidity was recorded for both the grainages and impact of these weather parameters on reproductive performance of *A. mylitta* was studied by correlating fecundity, hatching % and egg retention with daily temperature and relative humidity.

**Statistical analysis:** Statistical analysis was done using analysis of variance and means were compared by using Duncan's Multiple Range Test (DMRT).

## RESULTS AND DISCUSSION

### Fecundity

There was a significant difference in the fecundity observed across the different days of female emergence during first grainage period. Observed fecundity was ranged from 111 to 219 eggs/moth. Highest fecundity of 219 eggs/female was recorded at 10<sup>th</sup> day of grainage and it was followed by 13<sup>th</sup> the (218), 14<sup>th</sup> day (216) 20<sup>th</sup> day (207.30) and 11<sup>th</sup> day (206.80) and fecundity of these days were on par with each other. Similarly next better fecundity was recorded between 3<sup>rd</sup> to 6<sup>th</sup> days and 18<sup>th</sup> to 21<sup>st</sup> days of emergence. Fecundity recorded during these days was also statistically on par with fecundity recorded during 10<sup>th</sup> to 15<sup>th</sup> days of emergence, However marginal difference was observed between these periods. Similarly optimum range of fecundity (152.50 - 173.60 eggs/female) was observed on remaining days except 7<sup>th</sup> and 8<sup>th</sup> days of emergence where fecundity was lowest as compared to all other emergence period (Table 1).

Similar observations were made during second grainage as well to understand whether pattern of reproductive performance follows the pattern of first grainage. Results revealed that there was a dissimilar pattern of fecundity was observed across the emergence period. Recorded fecundity among the different days of emergence was statistically significant similar to first grainage. However the pattern of fecundity across different days was

differed from the first grainage. Overall fecundity among all the day ranged from 166.90 to 224.30 eggs per female. Highest fecundity was recorded on 13<sup>th</sup> day of emergence (224.30) and it was significantly differed from remaining days; 16<sup>th</sup> (221), 11<sup>th</sup> (216.40), 2<sup>nd</sup> (216), 17<sup>th</sup> (210.30), 7<sup>th</sup> (208.10), 14<sup>th</sup> (206.80) 1<sup>st</sup> (196.40) and 8<sup>th</sup> (194.60). Lowest fecundity was observed at 4<sup>th</sup> (166.90) whereas on remaining days average fecundity was observed (Table 2).

Overall data of fecundity over different days of female emergence found fluctuating in both the seasons. Fluctuation in the egg laying capacity of tasar silkworm might be due to various factors like, lack of sufficient nutrients available during rearing period and also unseasonal emergence affects the reproductive performance (Chaudhary, 1996). Sahu (2004) reported that grainage performance during the pre-seed and seed corps suffer due to low humidity and low temperature and high temperature resulting in requirement of more number of cocoons for producing DFLs, poor moth quality and low to very low hatching (<25%) in muga silkworm. Similar results were obtained during second grainage. However these results are not in line with grainage performance of first season. Variations in the finding might be due to the difference in the seasonal difference during the rearing and adult emergence.

### Hatching

Hatching varied from 70.79 to 93.31 per cent during first grainage. Highest hatching was recorded on first day (93.31%), followed by 7<sup>th</sup> (90.95%), 12<sup>th</sup> (90.18%) and 6<sup>th</sup> (90.14%) days of emergence and they were statistically on par with each other. Lowest hatching (70.79 %) was recorded on 8<sup>th</sup> and 13<sup>th</sup> days of emergence with 70.80 and 71.32 per cent respectively. These were on par with 9<sup>th</sup> (72.51%), 20<sup>th</sup> (72.15%), 11<sup>th</sup> (72.90%), 23<sup>rd</sup> (74.02%), 16<sup>th</sup> (74.06%) and 21<sup>st</sup> (74.46%) day of emergence. While, on remaining days fecundity was 78.72 to 86.68 per cent (Table 1).

During second grainage also, varied hatching was recorded across the days of emergence. Out of 17 days of emergence moths emerged on 12<sup>th</sup> day

recorded maximum hatching (95.24%) followed by the moths emerged on second day (94.00%) of hatching and were on par with each other. Lowest hatching was recorded from the moths emerged on 6<sup>th</sup> (85.02%) followed by 5<sup>th</sup> (85.51%), 16<sup>th</sup> (86.92%), 14<sup>th</sup> (87.26%) and 1<sup>st</sup> day (87.91%). On remaining days hatching ranged from 88.84 to 91.62 per cent (Table 2).

Generally temperature and relative humidity play major role in the hatching. Usually egg desiccation will occur due to higher temperature. During the present study temperature has shown negative correlation with hatching percentage and wet temperature and relative humidity have shown

positive significant relationship with hatching percentage during first grainage. These findings are in line with findings of Kovalev (1970), Ayuzawa *et al.* (1972), Jolly (1983), Narashimhanna (1988), Biram Saheb and Gowda (1987) and Ming (1994). However in the second grainage wet temperature and relative humidity have shown negative correlation that might be due to seasonal difference.

### Egg retention (%)

Results revealed that there was no significant difference across the different emergence days. However data on the unlaidd eggs revealed certain marginal difference over emergence period. Among

Table 1. Effect of seasonal adult emergence on reproductive performance of tasar silk moth during first grainage

Days of emergence	Fecundity*	Hatching**	Egg retention**	Hatching % in retained eggs**
1	173.60 (13.19) <sup>a-d</sup>	93.32 (74.99) <sup>a</sup>	13.95 (21.92) <sup>ab</sup>	4.50 (12.24) <sup>ab</sup>
2	166.10 (12.91) <sup>a-d</sup>	86.68 (68.57) <sup>c</sup>	29.25 (32.73) <sup>a</sup>	4.91 (12.80) <sup>ab</sup>
3	187.90 (13.73) <sup>a-d</sup>	85.66 (67.72) <sup>cd</sup>	6.80 (15.11) <sup>b</sup>	1.75 (7.61) <sup>abc</sup>
4	172.90 (13.17) <sup>a-d</sup>	85.97 (67.98) <sup>cd</sup>	10.55 (18.95) <sup>ab</sup>	3.52 (10.82) <sup>abc</sup>
5	187.20 (13.70) <sup>a-d</sup>	81.91 (64.80) <sup>def</sup>	13.52 (21.57) <sup>ab</sup>	0.73 (4.89) <sup>cd</sup>
6	193.30 (13.92) <sup>abc</sup>	90.14 (71.67) <sup>b</sup>	16.56 (24.00) <sup>ab</sup>	0.00 (0.00) <sup>d</sup>
7	111.90 (10.60) <sup>e</sup>	90.96 (72.47) <sup>ab</sup>	14.12 (22.06) <sup>ab</sup>	0.00 (0.00) <sup>d</sup>
8	138.40 (11.79) <sup>de</sup>	71.32 (57.60) <sup>h</sup>	21.53 (27.64) <sup>ab</sup>	6.00 (14.47) <sup>a</sup>
9	169.00 (13.02) <sup>a-d</sup>	72.51 (58.35) <sup>h</sup>	10.52 (18.91) <sup>ab</sup>	1.29 (6.52) <sup>cbd</sup>
10	219.60 (14.84) <sup>a-d</sup>	85.49 (67.58) <sup>cd</sup>	10.88 (19.25) <sup>ab</sup>	0.00 (0.00) <sup>d</sup>
11	206.80 (14.40) <sup>abc</sup>	72.90 (58.61) <sup>h</sup>	6.46 (14.72) <sup>b</sup>	0.00 (0.00) <sup>d</sup>
12	195.00 (13.98) <sup>abc</sup>	90.18 (71.71) <sup>b</sup>	14.87 (22.67) <sup>ab</sup>	0.00 (0.0) <sup>d</sup>
13	218.70 (14.81) <sup>a</sup>	70.80 (57.27) <sup>h</sup>	17.75 (24.91) <sup>ab</sup>	0.00 (0.00) <sup>d</sup>
14	216.50 (14.73) <sup>ab</sup>	78.73 (62.51) <sup>fg</sup>	13.29 (21.37) <sup>ab</sup>	0.00 (0.00) <sup>d</sup>
15	196.70 (14.04) <sup>abc</sup>	81.82 (64.73) <sup>def</sup>	14.00 (21.96) <sup>ab</sup>	0.00 (0.00) <sup>d</sup>
16	168.50 (13.00) <sup>a-d</sup>	74.06 (59.36) <sup>h</sup>	28.35 (32.16) <sup>a</sup>	0.00 (0.00) <sup>d</sup>
17	169.10 (13.02) <sup>a-d</sup>	83.95 (66.36) <sup>cde</sup>	14.21 (22.13) <sup>ab</sup>	4.50 (12.24) <sup>ab</sup>
18	185.80 (13.65) <sup>a-d</sup>	81.18 (64.26) <sup>ef</sup>	11.30 (19.64) <sup>ab</sup>	0.00 (0.00) <sup>d</sup>
19	186.80 (13.69) <sup>a-d</sup>	82.74 (65.42) <sup>c-f</sup>	10.78 (19.16) <sup>ab</sup>	0.00 (0.00) <sup>d</sup>
20	207.30 (14.42) <sup>abc</sup>	72.15 (58.13) <sup>h</sup>	17.13 (24.44) <sup>ab</sup>	0.00 (0.00) <sup>d</sup>
21	180.70 (13.46) <sup>a-d</sup>	74.46 (59.62) <sup>gh</sup>	16.20 (23.72) <sup>ab</sup>	0.00 (0.00) <sup>d</sup>
22	161.00 (12.71) <sup>bcd</sup>	80.54 (63.80) <sup>ef</sup>	16.63 (24.06) <sup>ab</sup>	0.00 (0.00) <sup>d</sup>
23	152.50 (12.37) <sup>cd</sup>	74.03 (59.34) <sup>h</sup>	27.00 (31.29) <sup>a</sup>	0.00 (0.00) <sup>d</sup>

Values are means of 10 replications; Means followed by same letter in the column do not differ significantly by DMRT (P=0.01); Figures in parentheses are square root\* and arcsine\*\* transformed values



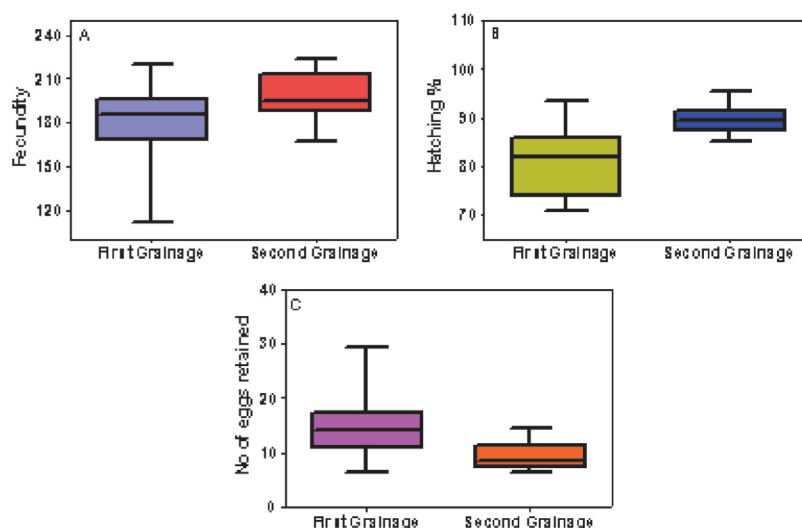


Fig. 1 Variability in the reproductive parameters of *A. mylitta* during first and second grainage periods: A) Fecundity, B) Hatching %, C) Egg retention

the different days of emergence maximum egg retention was recorded on 2<sup>nd</sup> day (29.25 %), which was statistically on par with 16<sup>th</sup> (28.35 %) and 23<sup>rd</sup> day (27.00 %). Whereas, minimum eggs retention was recorded on 11<sup>th</sup> day (6.40 %) of emergence followed by 3<sup>rd</sup> (6.80%) and 9<sup>th</sup> day (10.52%) of emergence and these were on par with each other. On remaining days of emergence intermediate range (27.64 to 18.91) of egg retention was observed (Table 1).

With respect to number of eggs retained significant difference was observed among the different days of moth emergence during second grainage. Maximum eggs were retained in the moths which are emerged on 17<sup>th</sup> day (14.39 %) followed by 12<sup>th</sup> (12.81 %) day of emergence and it was on par with 1<sup>st</sup>, 3<sup>rd</sup>, 5<sup>th</sup>, 6<sup>th</sup>, 8<sup>th</sup>, 13<sup>th</sup> and 15<sup>th</sup> days of emergence (21.70 %). Minimum was noticed during 11<sup>th</sup> day (6.49 %) and it was on par with remaining days (Table 2).

Whole data of per cent egg retention from both the seasons ranged from 6.46 to 29.25. Further it was observed that egg retention had a positive correlation with temperature and relative humidity during first grainage. Similar results were obtained by Mathur *et al.* (1995), where they reported that

high temperature with high humidity favoured high retention of eggs in the ovary. During the second grainage results were in contrast to first grainage it might be due to seasonal difference in rearing, no diapause period and adult emergence season.

### Hatching in retained eggs

In the study whether retained eggs are fertile or unfertile, results revealed that most of the retained eggs were unfertile with less than 2 per cent of hatching across all the days of emergence. Among the different days of emergence negligible per cent of hatching was observed on 2<sup>nd</sup> (1.40%), 9<sup>th</sup> (1.18%), 8<sup>th</sup> (1.15), 4<sup>th</sup> (1.09%), 3<sup>rd</sup> (0.76), 1<sup>st</sup> (0.72%) and 17<sup>th</sup> (0.39) day emerged moths. No hatching was observed in remaining days of moth emergence. However, during second grainage hatching per cent in retained eggs was completely nil across all the days of emergence (Table 1).

Poor hatching in the retained eggs (it was almost nil except few days in first grainage) might be due to incomplete development of the ovum, insufficient semen and due to higher temperature. Since higher temperature desiccation of eggs will take place which ultimately leads to unfertile eggs. Presently there were no studies in same line hence results

Table 2. Effect of seasonal adult emergence on reproductive performance of tasar silk moth during second grainage

Days of emergence	Fecundity*	Hatching**	Egg retention**	Hatching % in retained eggs**
1	196.40(14.03) <sup>abc</sup>	87.91 (69.63) <sup>c-f</sup>	8.38 (16.82) <sup>ab</sup>	0.00
2	216.00 (14.71) <sup>ab</sup>	94.00 (75.59) <sup>ab</sup>	7.09 (15.44) <sup>b</sup>	0.00
3	184.20 (13.59) <sup>bc</sup>	90.03 (71.57) <sup>cde</sup>	11.76 (20.04) <sup>ab</sup>	0.00
4	166.90 (12.94) <sup>c</sup>	91.48 (73.01) <sup>bc</sup>	7.87 (16.28) <sup>ab</sup>	0.00
5	192.00 (13.87) <sup>abc</sup>	85.50 (67.60) <sup>ef</sup>	9.59 (18.03) <sup>ab</sup>	0.00
6	185.40 (13.63) <sup>bc</sup>	85.01 (67.20) <sup>f</sup>	9.38 (17.83) <sup>ab</sup>	0.00
7	208.10 (14.44) <sup>ab</sup>	88.84 (70.46) <sup>cde</sup>	7.58 (15.97) <sup>b</sup>	0.00
8	194.60 (13.97) <sup>abc</sup>	89.76 (71.13) <sup>cde</sup>	9.41 (17.85) <sup>ab</sup>	0.00
9	186.30 (13.67) <sup>bc</sup>	91.04 (72.56) <sup>cde</sup>	7.02 (15.36) <sup>b</sup>	0.00
10	189.6 (13.79) <sup>abc</sup>	89.63 (71.19) <sup>c-f</sup>	8.12 (16.55) <sup>ab</sup>	0.00
11	216.40 (14.73) <sup>abc</sup>	89.55 (71.11) <sup>c-f</sup>	6.49 (14.57) <sup>b</sup>	0.00
12	189.00 (13.77) <sup>a</sup>	95.24 (77.37) <sup>a</sup>	12.81 (20.97) <sup>ab</sup>	0.00
13	224.30 (14.99) <sup>a</sup>	90.99 (72.51) <sup>bcd</sup>	10.99 (19.35) <sup>ab</sup>	0.00
14	206.80 (14.40) <sup>ab</sup>	87.26 (69.06) <sup>c-f</sup>	8.17 (16.06) <sup>b</sup>	0.00
15	189.40 (13.79) <sup>abc</sup>	91.61 (73.14) <sup>bc</sup>	11.53 (19.84) <sup>ab</sup>	0.00
16	221.90 (14.91) <sup>a</sup>	86.92 (68.77) <sup>def</sup>	7.03 (15.37) <sup>b</sup>	0.00
17	210.30 (14.52) <sup>ab</sup>	89.11 (70.71) <sup>c-f</sup>	14.39 (22.29) <sup>a</sup>	0.00

Values are means of 10 replications; Means followed by same letter in the column do not differ significantly by DMRT (P=0.01); Figures in parentheses are square root\* and arcsine\*\* transformed values

Table 3. Influence of weather parameters on reproductive parameters of tasar silkworm

Reproduction parameters	First grainage		Second grainage	
	Temperature	Relative Humidity	Temperature	Relative Humidity
Fecundity (Eggs/female)	-0.289	-0.020	-0.420	-0.174
Hatching (%)	-0.286	0.284	-0.096	-0.170
Egg retention	0.068	0.176	-0.574*	-0.242

\*. Correlation is significant

cannot be discussed further. When comparison of fecundity, hatching and egg retention was made between the both grainage periods, it was found that reproductive performance of the moths emerged during second grainage was superior over first grainage moths (Fig. 1). This variability might be due to the reason that first grainage moths are gone through pupal diapause, since diapause known to adversely affect reproductive physiology in some lepidopterans (Dillon and Hasan, 2018).

Correlation analysis between reproductive parameters and weather parameters during first grainage revealed that none of the reproductive parameter had a significant correlation with any of the three parameters considered (fecundity, hatching % and retained eggs). However there was a non-significant negative correlation was observed between temperature and fecundity ( $r=-0.289$ ), similarly hatching per cent also showed non-significant negative correlation ( $r=-0.286$ ), whereas

egg retention showed positive and non-significant relationship ( $r=0.068$ ) with temperature. While, relative humidity found to have non-significant negative correlation with fecundity ( $r=-0.020$ ) and positive and non-significant relationship with hatching per cent ( $r=0.284$ ) and egg retention ( $r=0.176$ ) (Table 3).

Similar analysis was also carried out during second grainage between reproductive parameters and weather parameters all three parameters (Fecundity, Hatching % and Retained eggs) showed negative relationship with temperature and relative humidity. However there was a non-significant negative correlation was observed between temperature and fecundity ( $r=-0.289$ ), similarly hatching per cent also showed non-significant negative correlation ( $r=-0.286$ ), whereas egg retention showed negative and significant relationship ( $r=-0.574$ ) with temperature. Similarly relative humidity found to have non-significant negative correlation with fecundity ( $r=-0.174$ ) hatching per cent ( $r=-0.170$ ) and egg retention ( $r=-0.242$ ) (Table 3). These results indicates that prevailing temperature and relative humidity during emergence period do don't have major influence over fecundity, hatching percentage and egg retention, rather temperature and relative humidity during the cocoon storage will impact these reproductive parameters as reported by Mathur *et al.* (1995) and Sahu (2004).

Based on the outcome of the present study it is evident that moths emerging across the different days during grainage period are poses significant difference in their reproductive potential and in reproductive biology. However we cannot consider any particular day or a period during the process of emergence for better reproductive traits, since they were scattered randomly across the seasonal emergence period. Further indicating that adult emergence has nothing to do with reproductive biology of *A. mylitta*. Experimental results of present study also signify that temperature and relative humidity during emergence period are not major influencers on reproductive biology of the tasar silkmoth. Considering these outcomes it seems that weather parameters during rearing period and cocoon storage period along with quality of foliage

supplied during the larval stage are major factors influencing the reproductive performance of tasar silkmoth.

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## Diversity of Hemipteran families at Agri-biodiversity park, Hyderabad, India

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**ABSTRACT:** The diversity and abundance of Hemipteran families at Agri-biodiversity park of Professor Jayashankar Telangana State Agricultural University, Hyderabad, Telangana, India was studied from September 2019 to January 2020. A total of 12,575 individuals under 22 families of Hemiptera were recorded by using five different collection methods viz. pitfall trap, yellow pan trap, manual collection, light trap and yellow sticky trap. Family Cicadellidae was found to be the most abundant family (RA=32.70%), followed by Aleyrodidae (RA=12.47%) and Delphacidae (RA=12.30%), while Eurybrachidae (RA=0.10%), Flatidae (RA=0.10%) and Scutelleridae (RA=0.11%) were the least abundant families. Among the five different collection methods, light trap recorded the maximum number of individuals (6010) followed by yellow sticky trap (3815) whereas, manual collection method (313) recorded the least number of individuals. The Shannon-Weiner diversity Index, Margalef's species richness index and Pielou's evenness index for the Hemipteran fauna of the study area were 2.252, 2.225 and 0.728 respectively. © 2021 Association for Advancement of Entomology

**KEYWORDS:** Hemiptera, relative abundance, Shannon-Weiner diversity Index, Margalef's species richness index, Pielou's evenness index

### INTRODUCTION

Hemipterans, commonly called bugs, are the most diverse group among the exopterygote insects. They are mostly plant sap suckers and vectors of many viral and phytoplasmal plant diseases. Some are also predators of other insects and some are inhabitants of aquatic ecosystem. There are 103,590 species of Hemipteran under 152 families and four suborders known worldwide. The Indian Hemipteran fauna is represented by 6479 species

under 92 families (ZSI, 2012; Chandra, 2011). Professor Jayashankar Telangana State Agricultural University (PJTSAU), Hyderabad, is the first Agricultural University in India to initiate the establishment of Agri-Biodiversity Park (ABP) in August 2008, in 60 ha area with natural ecosystem and half of it is occupied by a pond (Khan and Krishna, 2017). The existing flora of this habitat includes tree species like *Tectona grandis* Linn. f., *Butea monosperma* (Lam.) Taub., *Syzygium cumini* (L.) Skeels, *Ficus* spp. (L.), *Millettia*

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*pinnata* (L.) Panigrahi, *Madhuca longifolia* (J.Konig) J.F.Macbr., *Albizia lebbeck* (L.) Benth., *Cassia* spp. (L.), *Dalbergia sissoo* Roxb., *Vachellia nilotica* (L.) P.J.H.Hurter & Mabb., *Tamarindus indica* L., *Annona reticulata* L., *Azadirachta indica* A.Juss. and *Prosopis juliflora* (Sw.) DC., besides a diverse species of shrubs, herbs and grasses. There was no earlier documentation of the Hemipteran fauna from this habitat, hence the present investigation was taken up.

## MATERIALS AND METHODS

**Study site:** The sampling of hemipterans was carried out from September 2019 to January 2020 at the Agri-biodiversity Park of PJTSAU, Rajendranagar, Hyderabad, which is located at 17°18' N and 78°24' E and an altitude of 559 m from mean sea level.

**Collection methods:** The collection of hemipteran fauna was carried out at weekly intervals using five different sampling methods: (i) pitfall traps (N=50), (ii) yellow pan traps (N=30), (iii) light traps (N=5), (iv) yellow sticky traps (N=30) and (v) manual collection.

Soap water was used in pitfall traps (transparent plastic cups of 8 cm top diameter and 10 cm height) and yellow pan traps (bright yellow-colored plastic basins with 18 cm diameter and 3 cm depth) to kill the trapped insects. The traps were inspected in 24 hours (the next day) and the trapped insects were collected and preserved in containers with 70% alcohol. Manual collection was done every week by 3 hours of random active sweepings during the day time (9 am-12 noon) with a sweep net of 30 cm hoop diameter and 80 cm handle length. A cotton swab dipped in ethyl acetate was used to anesthetize the collected insects. Light traps fitted with collecting bottles (containing 50% alcohol) were operated in evening hours (6 to 9 pm) to collect nocturnal insects. Yellow sticky traps were also inspected in 24 hours and the trapped insects were counted directly with the help of a magnifying lens. The specimens were identified up to family level with help of the key by Triplehorn and Johnson (2005).

**Statistical analysis:** Shannon-Wiener Diversity index, Margalef's species richness index and Pielou's Evenness Index were computed by using the software; PAST (Paleontological Statistics Tool) version 3.25. The relative abundance of each Hemipteran family was calculated by the following formula.

$$\text{Relative abundance (\%)} = \frac{n_i \times 100}{N}$$

Where, N: the total number of individuals in all families

$n_i$ : the number of individuals in  $i^{\text{th}}$  family

## RESULTS AND DISCUSSION

A total of 12,575 individuals belonging to 22 families of Hemiptera were collected. According to the number of individuals collected; following trend was observed among different hemipteran families Cicadellidae (4112) > Aleyrodidae (1568) > Delphacidae (1547) > Aphididae (1199) > Lygaeidae (766) > Cydnidae (673) > Pentatomidae (626) > Miridae (474) > Coreidae (404) > Anthocoridae (337) > Tingidae (172) > Reduviidae (143) > Corixidae (130) > Veliidae (103) > Hydrometridae (76) > Cercopidae (72) > Membracidae (67) > Pyrrhocoridae (42) > Dictyopharidae (26) > Scutelleridae (14) > Eurybrachidae = Flatidae (12) (Table 1).

Out of total 12,575 individuals collected, light trap recorded maximum number of hemipterans with 47.79 per cent (6010 individuals) followed by yellow sticky trap with 30.34 per cent (3815), yellow pan trap with 14.29 per cent (1797) and pitfall trap (5.09%). Manual collection method recorded least with 2.49 per cent (313) (Fig. 1). In terms of number of families, yellow pan trap recorded the maximum number of families (14 families) followed by manual collection and light Trap (13 families each), while yellow sticky trap and pitfall trap recorded the minimum number of families (nine families each). The highest number of hemipterans in light trap was mainly because of major share of leaf hoppers (Cicadellidae) and plant hoppers (Delphacidae). Yellow sticky trap recorded second highest catch in number because of aphids (Aphididae) and



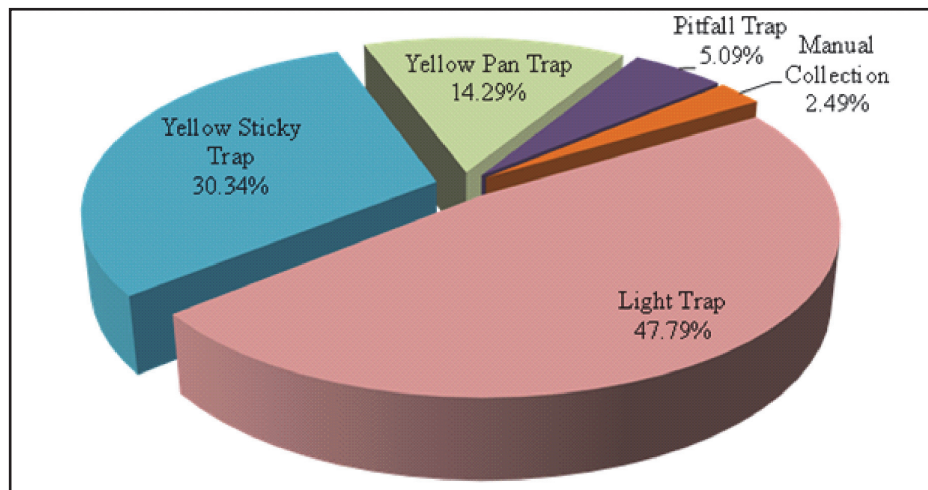


Fig. 1. Percentage of Hemipterans recorded in different collection methods

whiteflies (Aleyrodidae). Manual collection method recorded most of the heteropterans and light traps recorded some aquatic hemipterans along with most Lygaeidae. It is not easy to infer that any particular trap is effective for collecting hemipterans because different families were recorded in different traps. A combination of trapping methods can be used for collecting diversity of hemipterans. However, yellow sticky trap may not be effective, if identification is to be done beyond family level because of difficulty in recovering the trapped insects from the glue.

In earlier studies by Chandra *et al.* (2012), nine families of hemipterans (Pentatomidae, Reduviidae, Lygaeidae, Pyrrhocoridae, Alydidae, Cercopidae, Coreidae, Cydnidae and Dinidoridae) were documented from Veerangana Durgavati Wildlife Sanctuary of Madhya Pradesh. Chandra and Kushwaha (2013a, b) recorded 10 families of Hemipterans (Pentatomidae, Reduviidae, Alydidae, Lygaeidae, Pyrrhocoridae, Coreidae, Dictyopharidae, Asopinae, Cydnidae and Dinidoridae) from Kheoni Wildlife Sanctuary and 13 families of Hemipterans (Cercopidae, Aphrophoridae, Dictyopharidae, Reduviidae, Coreidae, Lygaeidae, Pyrrhocoridae, Pentatomidae, Alydidae, Asopinae, Cydnidae, Dinidoridae, Scutelleridae) from Singhori Wildlife Sanctuary, Madhya Pradesh. Kalita *et al.* (2014) recorded 18 species under 12 families of hemipterans from

Jorhat district of Assam. Chandra *et al.* (2015 a, b) recorded 11 families of hemipterans (Reduviidae, Pentatomidae, Coreidae, Lygaeidae, Scutelleridae, Alydidae, Largidae, Pyrrhocoridae, Plataspidae, Cydnidae, Dinidoridae) from Ralamandal Wildlife Sanctuary and 13 families (Reduviidae, Pentatomidae, Coreidae, Lygaeidae, Scutelleridae, Alydidae, Largidae, Pyrrhocoridae, Cercopidae, Nabidae, Asopinae, Cydnidae, Dinidoridae) from Ratapani wildlife sanctuary of Madhya Pradesh. Similarly, Chandra *et al.* (2017) collected 187 specimens and reported 50 species under 11 families (Pentatomidae, Reduviidae, Cydnidae, Alydidae, Coreidae, Pyrrhocoridae, Lygaeidae, Cercopidae, Nabidae, Membracidae, Ricaniidae) of Hemiptera from Daman and Diu.

Among the 22 recorded families of Hemiptera, family Cicadellidae was the most abundant (RA=32.70%), followed by Aleyrodidae (RA=12.47%) and Delphacidae (RA=12.30%), while Eurybrachidae (RA=0.10%), Flatidae (RA=0.10%) and Scutelleridae (RA=0.11%) were the least abundant families. Cicadellidae, Aleyrodidae, Delphacidae, Aphididae, Lygaeidae and Cydnidae were highly abundant (RA > 5%). Pentatomidae, Miridae, Coreidae, Anthocoridae, Tingidae, Reduviidae and Corixidae were moderately abundant (RA=1-5%). While Veliidae, Hydrometridae, Cercopidae, Membracidae, Pyrrhocoridae, Dictyopharidae, Scutelleridae,

Table 1. Number of individuals and Relative Abundance of Hemipteran families in different collection methods

No.	Families	Total No.	RA (%)	Collection methods
1	Aleyrodidae	1568	12.47	YST
2	Anthocoridae	337	2.68	YPT, LT, YST
3	Aphididae	1199	9.53	YPT, YST
4	Cercopidae	72	0.57	YPT, PT, MC
5	Cicadellidae	4112	32.70	YPT, PT, MC, LT, YST
6	Coreidae	404	3.21	YPT, PT, MC, LT, YST
7	Corixidae	130	1.03	LT
8	Cydnidae	673	5.35	YPT, PT, LT
9	Delphacidae	1547	12.30	YPT, PT, MC, LT, YST
10	Dictyopharidae	26	0.21	LT
11	Eurybrachidae	12	0.10	MC
12	Flatidae	12	0.10	MC
13	Hydrometridae	76	0.60	MC, LT
14	Lygaeidae	766	6.09	YPT, PT, LT, YST
15	Membracidae	67	0.53	YPT, MC
16	Miridae	474	3.77	YPT, PT, MC, LT, YST
17	Pentatomidae	626	4.98	YPT, PT, MC, LT, YST
18	Pyrrohocoridae	42	0.33	YPT, MC
19	Reduviidae	143	1.14	YPT, PT, MC
20	Scutelleridae	14	0.11	MC
21	Tingidae	172	1.37	YPT, LT
22	Veliidae	103	0.82	LT
	Total	12575		

LT- Light trap; MC- Manual collection method; PT- Pitfall trap; YPT- Yellow pan trap; YST- Yellow sticky trap

Eurybrachidae and Flatidae were less abundant (RA < 1%) (Table 1).

Hemipterans are mostly plant sap sucking insects and many are serious pests of agricultural crops. The phytophagous families recorded in the present study include agricultural pests from Cicadellidae, Aleyrodidae, Delphacidae, Aphididae, Lygaeidae, Tingidae, Membracidae, Pentatomidae and Pyrrhocoridae. Besides these, Dictyopharidae, Scutelleridae, Cercopidae, Eurybrachidae and Flatidae also have phytophagous members but are less damaging. Cicadellidae and Delphacidae collectively accounted for 45% of the total hemipterans. Whiteflies were found to be the second most abundant group even though they were

collected by only one method of collection i.e. yellow sticky trap. Aphids (Aphididae) were collected by two methods viz. yellow pan trap and yellow sticky trap and ranked 4<sup>th</sup> in terms of abundance (RA= 9.53%). Family Lygaeidae (commonly called as seed bugs) is the 5<sup>th</sup> most abundant family (RA= 6.09%). Some members of family Lygaeidae are phytophagous, some inhabit the ground and leaf litter and some are nocturnal. They were collected by four different collection methods viz., pitfall trap, yellow pan trap, light trap and yellow sticky trap. It was followed by family Cydnidae in terms of abundance (RA= 5.35). Family Cydnidae are called as burrowing bugs as they remain in soil burrows. They can also feed on the plant roots but are not much harmful. It is one

of the extensively occurring families next to Pentatomidae under the superfamily Pentatomoidea. There are 72 species under 28 genera recorded from India so far (Biswas, 2013). They were collected in large numbers in pitfall trap, light trap and yellow pan trap after rainfalls.

Some families are also having predators of other insects acting as pests of crops, which includes; Reduviidae, Anthocoridae, Miridae and Pentatomidae. During the study three families of aquatic hemipterans were recorded viz., Corixidae, Veliidae and Hydrometridae. All these three families were recorded from the light trap. The pond present in the area is the reason for their occurrence. Barman and Deka (2015) reported 15 species of aquatic hemipterans under 8 families from Ghaga Beel of Nalbari district of Assam. Vssou *et al.* (2017) reported 6 families of aquatic hemipterans from Sengunam pond, Perambalur, Tiruchirappalli, Tamil Nadu. Aquatic insects play an important role in aquatic food chain and help in nutrient recycling. Besides this, they also act as ecological indicators in the aquatic ecosystems (Vasantkumar and Roopa, 2014).

The Shannon-Weiner diversity, Margalef's species richness and Pielou's evenness indices for the hemipteran fauna of the study area were 2.252, 2.225 and 0.728, respectively, indicating their good diversity in the study area. Vegetation structure and flower abundance are key factors for species richness, abundance and species composition of bugs (Zurbrugg and Frank, 2006). A good vegetation cover throughout the study period can be the reason for the diversity of hemipterans in this area. The present study records the hemipteran fauna for the first time from this area and it provides a preliminary data, which will be helpful for future works focusing on individual hemipteran families and their identification up to species level.

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## Seasonal foraging activity of stingless bee *Tetragonula travancorica* Shanas and Faseeh (Hymenoptera: Apidae: Meliponini)

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**ABSTRACT:** The foraging hive activity of stingless bee *Tetragonula travancorica* Shanas and Faseeh was studied from November 2018 to August 2019. The activity varied between the seasons, weather conditions and time hours of study. The outgoing and incoming pollen foragers exhibited two peaks in activity, from 0800-1200 h (first) and during 1500-1600 h (second). The activity of incoming non-pollen foragers displayed only one distinct peak between 1000-1200 h except during the south-west monsoon period. The greatest activity was recorded during the dry season (January-May), followed by the south-west monsoon (June-August) and north-east monsoon (November- December) seasons. Maximum overall activity was recorded during hotter months February, March and April while the lowest was observed in January and December. At any season or time, the number of incoming foragers without pollen was greater than pollen foragers.

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**KEYWORDS:** *Tetragonula travancorica*, outgoing, incoming, foragers, pollen

### INTRODUCTION

Stingless bees are eusocial insects that are considered to be the smallest honey-producing bees found in tropical and subtropical regions around the world. The social stingless bees (Meliponini) are globally more diverse than *Apis*, with approximately 500 species (Engel *et al.*, 2018) among which, the genus *Tetragonula* Moure, 1961 is the most speciose with 31 valid species (Rasmussen *et al.*, 2017; Engel *et al.*, 2018). The genus *Tetragonula* is the most abundant in the Indo- Malayan region (Moure, 1961; Velthuis, 1997). Shanas and Faseeh

(2019) provided keys to the species of *Tetragonula* of the Indian subcontinent and described three new species of stingless bees viz., *T. travancorica*, *T. calophyllae* and *T. perlucipinnae* from south India. Stingless bees are also called dammer bees since they collect dammer from dipterocarp trees and mix with the wax they produce to build their nests (Rasmussen, 2013). The term stingless bees denote the presence of a vestigial sting that cannot inflict pain in humans. The defence mechanism is marked by the biting with modified powerful mandibles or production of a caustic agent in some species irritating the enemy (Makkar *et al.*, 2018).

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Stingless bees serve as pollinators for a large portion of tropical plant species which constitutes one-fifth of local angiosperms (Wilms *et al.*, 1996) as well as economically important crops (Heard, 1999). Foraging activity of the worker bee population is very crucial to the perpetuation of the colony in a healthy state and the number of outgoing bees per unit time indicates the strength of the colony (Mattu and Verma, 1985). Considering the facts, proper knowledge about the foraging activity of the colony is essential to maintain the efficiency of controlled pollination using stingless bees and also honey production. Hence foraging studies on *T. travancorica* Shanas and Faseeh were conducted and results are presented in this paper.

## MATERIALS AND METHODS

**Study site:** The present study was conducted on the campus of College of Agriculture, Vellayani, in district Thiruvananthapuram, Kerala which is located at 8° 25' 47" north latitude and 76° 59' 7" east longitude and an altitude of 29 m above mean sea level. The location of the study is in a suburban region covering an area of 2.52 square km and has various agricultural, horticultural and natural ecosystems with diverse floral resources. The campus is bordered by Vellayani Lake on three sides, the only rainfed freshwater lake in the district. The study area comes under the tropical monsoon region experiencing the main part of its annual rainfall under the influence of south-west monsoon winds (800 to 1200 mm average rainfall) and lesser part during the north-east monsoon (450 to 500 mm average rainfall) period. The area also exhibits dry periods of winter and summer witnessing a very less amount of average rainfall ranging from 20 to 195 mm with an average daytime temperature varying between 22 to 25°C and 32 to 35°C respectively.

**Bee colonies:** Two stingless bee colonies of *T. travancorica* with uniform strength approx. 1500 worker bees maintained in wooden hives located 80 m apart were selected for observation. The stingless bee was identified based on the key provided in Shanas and Faseeh (2019). The measurements were taken under a stereoscopic microscope with the help of ocular micrometre.

**Collection of data on foraging activity:** The observations on foraging frequencies of worker bees were made from November 2018 to August 2019 at an interval of 14 days. The study period was divided into three seasons namely, North-east monsoon (November to December), Dry season (January to May) and South-west monsoon (June to August). The hive entrance was observed for 5 minutes at an hourly interval from morning 0600 to evening 1800 h with the help of a stopwatch (Bharath *et al.*, 2020; Jaapar *et al.*, 2018) and the mean value of recorded data for the twelve intervals was considered as foraging activity of the day.

**Statistical analysis:** The data were quantified in terms of the number of foragers leaving the hive eliminating the foragers with garbage load as 'outgoing foragers', the number of bees returning with pollen load as 'incoming pollen foragers' and without pollen load excluding the resin and mud foragers as 'incoming foragers without pollen'. The number of bees returning without pollen load was calculated by subtracting the number of bees carrying garbage from the total number of incoming foragers without pollen load. The term incoming without pollen loads indicates the bees carrying in nectar as well as water occasionally. Monthly and hourly mean of the data obtained was square-root transformed and classified accordingly into three seasons after which two factorial analysis was used to analyse the data.

## RESULTS

**Diurnal variation in foraging activity:** The foraging activity observed as per the number of outgoing foragers and incoming foragers were between 0630 and 1800 h during north-east monsoon season, 0600 and 1840 h during the dry season and 0610 and 1820 h during south-west monsoon season. The numbers of foragers active at different hours of the day and seasons differed statistically. The least number of outgoing foragers was seen during the early morning from 0600-0700 h. The activity gradually increased and reached a peak at 0900-1000 h thereafter declining slowly up to 1800 h (Table 1). Similar to outgoing foragers, the number of incoming foragers with pollen loads was



Table 1.Outgoing foragers irrespective of season

Months↓	Mean number of bees/5 minutes/colony - Hours →												Mean
	0600	0700	0800	0900	1000	1100	1200	1300	1400	1500	1600	1700	
November	3.27 (1.94)	8.42 (2.99)	33.16 (5.80)	29.68 (5.49)	14.96 (3.93)	20.23 (4.55)	16.68 (4.15)	11.72 (3.49)	11.87 (3.51)	14.56 (3.88)	8.23 (2.95)	4.72 (2.28)	14.79 (3.75 <sup>de</sup> )
December	1.00 (1.23)	3.20 (1.92)	28.13 (5.35)	23.15 (4.86)	13.96 (3.8)	22.39 (4.78)	12.95 (3.67)	9.23 (3.12)	11.20 (3.42)	12.40 (3.59)	5.98 (2.55)	2.73 (1.79)	12.19 (3.34 <sup>e</sup> )
January	1.00 (1.23)	1.42 (1.39)	11.79 (3.51)	27.38 (5.28)	24.16 (4.97)	11.30 (3.44)	19.42 (4.46)	15.90 (4.05)	8.06 (2.93)	7.87 (2.89)	7.83 (2.87)	5.77 (2.50)	11.82 (3.29 <sup>e</sup> )
February	1.60 (1.45)	18.41 (4.35)	56.84 (7.57)	48.51 (7.00)	31.21 (5.63)	30.64 (5.58)	27.48 (5.29)	16.52 (4.13)	17.71 (4.27)	13.83 (3.79)	18.01 (4.30)	7.48 (2.83)	24.02 (4.68 <sup>e</sup> )
March	1.00 (1.23)	30.45 (5.56)	34.97 (5.96)	48.93 (7.03)	56.08 (7.52)	41.56 (6.49)	31.96 (5.69)	22.09 (4.75)	27.06 (5.25)	24.27 (4.98)	15.65 (4.02)	11.66 (3.49)	28.81 (5.16 <sup>b</sup> )
April	10.73 (3.35)	32.89 (5.78)	49.84 (7.09)	55.03 (7.45)	45.77 (6.80)	38.40 (6.24)	34.61 (5.93)	33.61 (5.84)	32.93 (5.78)	26.86 (5.23)	27.71 (5.31)	18.90 (4.41)	33.94 (5.77 <sup>a</sup> )
May	9.75 (3.20)	25.32 (5.08)	29.12 (5.44)	39.99 (6.36)	29.08 (5.44)	30.24 (5.54)	24.30 (4.98)	14.48 (3.87)	25.10 (5.06)	19.53 (4.48)	14.77 (3.91)	13.32 (3.72)	22.92 (4.76 <sup>bc</sup> )
June	7.15 (2.77)	10.92 (3.38)	21.71 (4.71)	24.47 (4.99)	20.63 (4.59)	19.19 (4.44)	15.01 (3.94)	15.82 (4.04)	12.57 (3.62)	18.15 (4.32)	16.15 (4.1)	4.56 (2.25)	15.53 (3.93 <sup>d</sup> )
July	4.52 (2.24)	4.60 (2.26)	13.92 (3.79)	20.69 (4.60)	23.86 (4.94)	28.38 (5.37)	21.34 (4.67)	14.86 (3.92)	12.35 (3.59)	15.04 (3.94)	9.03 (3.1)	5.83 (2.51)	14.53 (3.74 <sup>de</sup> )
August	1.00 (1.23)	3.42 (1.98)	11.83 (3.51)	17.47 (4.24)	27.94 (5.33)	23.65 (4.91)	30.57 (5.57)	21.07 (4.64)	11.46 (3.46)	16.54 (4.13)	9.31 (3.13)	6.53 (2.65)	15.07 (3.73 <sup>de</sup> )
Mean	4.10 (1.98 <sup>e</sup> )	13.90 (3.47 <sup>e</sup> )	29.13 (5.27 <sup>ab</sup> )	33.53 (5.73 <sup>a</sup> )	28.77 (5.29 <sup>ab</sup> )	26.60 (5.13 <sup>b</sup> )	23.43 (4.83 <sup>b</sup> )	17.53 (4.18 <sup>c</sup> )	17.03 (4.09 <sup>cd</sup> )	16.90 (4.12 <sup>cd</sup> )	13.27 (3.62 <sup>de</sup> )	8.15 (2.84 <sup>f</sup> )	

Factors

Months (M)

Time (T)

M x T

SEm±

0.168

0.185

0.584

CD at 5%

0.469

0.514

Means in the columns/ rows with same alphabet do not differ significantly by DMRT at 5%

Figures in the parenthesis are  $\sqrt{(x + 0.5)}$  transformed values

Table 2. Incoming foragers with pollen irrespective of season

Months	Mean number of bees/5 minutes/colony - Hours →												Mean
	0600	0700	0800	0900	1000	1100	1200	1300	1400	1500	1600	1700	
November	1.00 (1.23)	2.39 (1.7)	15.72 (4.03)	18.74 (4.39)	13.56 (3.75)	7.15 (2.77)	2.90 (1.85)	2.70 (1.79)	1.47 (1.40)	2.18 (1.64)	1.00 (1.23)	1.00 (1.23)	5.82 (2.25 <sup>d</sup> )
December	1.00 (1.23)	1.47 (1.31)	13.73 (3.77)	13.33 (3.72)	9.42 (3.15)	5.98 (2.55)	3.97 (2.12)	3.92 (2.10)	2.24 (1.65)	2.09 (1.61)	1.47 (1.40)	1.23 (1.31)	4.99 (2.16 <sup>d</sup> )
January	1.00 (1.23)	1.00 (1.23)	4.42 (2.22)	14.84 (3.92)	10.82 (3.37)	4.55 (2.25)	3.39 (1.97)	3.08 (1.89)	2.39 (1.7)	3.10 (1.9)	2.83 (1.83)	2.35 (1.68)	4.48 (2.09 <sup>de</sup> )
February	1.00 (1.23)	3.42 (1.98)	19.30 (4.45)	23.76 (4.93)	11.90 (3.52)	13.07 (3.68)	4.89 (2.32)	6.22 (2.59)	4.66 (2.27)	3.57 (2.02)	1.87 (1.54)	1.42 (1.39)	7.92 (2.66 <sup>ab</sup> )
March	1.00 (1.23)	3.46 (1.99)	9.48 (3.16)	17.70 (4.27)	23.94 (4.94)	12.68 (3.63)	8.47 (2.99)	4.85 (2.31)	2.77 (1.81)	2.69 (1.79)	2.39 (1.7)	2.09 (1.61)	7.63 (2.62 <sup>bc</sup> )
April	1.47 (1.4)	3.62 (2.03)	18.10 (4.31)	25.26 (5.08)	21.43 (4.68)	10.87 (3.37)	6.10 (2.57)	6.22 (2.59)	4.47 (2.23)	3.21 (1.93)	2.51 (1.73)	1.60 (1.45)	8.74 (2.78 <sup>a</sup> )
May	1.00 (1.23)	4.81 (2.3)	7.54 (2.84)	5.15 (2.38)	4.49 (2.23)	4.08 (2.14)	2.90 (1.85)	3.08 (1.89)	2.39 (1.7)	1.68 (1.48)	1.95 (1.57)	1.00 (1.23)	3.34 (1.90 <sup>cd</sup> )
June	1.47 (1.4)	3.00 (1.87)	4.85 (2.31)	3.55 (2.01)	3.49 (1.99)	2.99 (1.87)	1.47 (1.4)	2.09 (1.61)	1.68 (1.48)	2.49 (1.73)	2.15 (1.63)	1.00 (1.23)	2.52 (1.71 <sup>i</sup> )
July	1.00 (1.23)	1.68 (1.48)	2.84 (1.83)	6.19 (2.59)	7.62 (2.85)	8.87 (3.06)	7.78 (2.88)	3.98 (2.12)	2.77 (1.81)	2.49 (1.73)	1.77 (1.51)	1.47 (1.4)	4.04 (2.04 <sup>def</sup> )
August	1.00 (1.23)	1.42 (1.39)	7.50 (2.83)	8.04 (2.92)	9.04 (3.09)	12.71 (3.63)	9.59 (3.18)	8.66 (3.03)	2.21 (1.65)	3.90 (2.1)	2.15 (1.63)	1.23 (1.31)	5.62 (2.33 <sup>bed</sup> )
Mean	1.09 (1.26 <sup>i</sup> )	2.63 (1.73 <sup>cd</sup> )	10.35 (3.17 <sup>bc</sup> )	13.65 (3.62 <sup>a</sup> )	11.57 (3.36 <sup>ab</sup> )	8.30 (2.89 <sup>c</sup> )	5.15 (2.31 <sup>d</sup> )	4.48 (2.19 <sup>d</sup> )	2.70 (1.77 <sup>e</sup> )	2.74 (1.79 <sup>e</sup> )	2.01 (1.57 <sup>ef</sup> )	1.44 (1.38 <sup>ef</sup> )	

Factors SEM± CD at 5%

Months (M)

Time (T)

M x T

0.121

0.132

0.418

0.336

0.368

1.162

Means in the columns/ rows with same alphabet do not differ significantly by DMRT at 5%

Figures in the parenthesis are  $\sqrt{(x + 0.5)}$  transformed values

Table 3. Incoming foragers without pollen irrespective of season

Months	Mean number of bees/5 minutes/colony - Hours →												Mean
	0600	0700	0800	0900	1000	1100	1200	1300	1400	1500	1600	1700	
November	1.68 (1.48)	6.75 (2.69)	5.67 (2.48)	8.66 (3.03)	11.67 (3.49)	15.43 (3.99)	16.96 (4.18)	13.67 (3.76)	15.68 (4.02)	12.19 (3.56)	9.47 (3.16)	6.99 (2.74)	10.40 (3.21 <sup>de</sup> )
December	1.00 (1.23)	2.48 (1.73)	5.56 (2.46)	8.23 (2.96)	9.39 (3.15)	17.22 (4.21)	17.73 (4.27)	11.71 (3.49)	10.97 (3.39)	10.17 (3.27)	12.21 (3.57)	5.98 (2.55)	9.39 (3.02 <sup>c</sup> )
January	1.00 (1.23)	1.00 (1.23)	5.41 (2.43)	15.42 (3.99)	13.47 (3.74)	11.51 (3.47)	18.44 (4.35)	10.90 (3.38)	9.04 (3.09)	9.75 (3.2)	7.36 (2.8)	5.28 (2.40)	9.05 (2.94 <sup>c</sup> )
February	1.42 (1.39)	14.36 (3.86)	31.83 (5.69)	28.35 (5.37)	22.86 (4.83)	27.91 (5.33)	21.36 (4.68)	19.73 (4.49)	17.03 (4.19)	18.28 (4.33)	19.28 (4.45)	9.35 (3.14)	19.31 (4.31 <sup>b</sup> )
March	1.00 (1.23)	25.28 (5.08)	23.06 (4.85)	25.83 (5.13)	38.56 (6.25)	30.07 (5.53)	23.69 (4.92)	21.93 (4.74)	24.55 (5.01)	22.63 (4.81)	17.55 (4.25)	17.36 (4.23)	22.62 (4.67 <sup>b</sup> )
April	7.11 (2.76)	20.98 (4.64)	27.06 (5.25)	32.64 (5.76)	41.28 (6.46)	35.52 (6.00)	30.55 (5.57)	30.40 (5.56)	24.90 (5.04)	26.22 (5.17)	22.78 (4.83)	23.83 (4.93)	26.94 (5.16 <sup>a</sup> )
May	4.45 (2.23)	17.20 (4.21)	22.33 (4.78)	30.83 (5.59)	26.34 (5.18)	31.94 (5.69)	29.06 (5.44)	25.56 (5.11)	23.03 (4.85)	18.93 (4.41)	15.52 (4.00)	9.54 (3.17)	21.23 (4.55 <sup>b</sup> )
June	4.49 (2.23)	9.89 (3.22)	14.90 (3.92)	21.19 (4.66)	19.13 (4.43)	14.08 (3.82)	11.89 (3.52)	17.58 (4.25)	15.76 (4.03)	17.28 (4.22)	15.27 (3.97)	6.30 (2.61)	13.98 (3.74 <sup>c</sup> )
July	1.68 (1.48)	5.01 (2.35)	7.33 (2.79)	13.27 (3.71)	20.55 (4.59)	21.25 (4.66)	19.89 (4.52)	19.69 (4.49)	16.58 (4.13)	11.31 (3.44)	9.12 (3.1)	6.38 (2.62)	12.67 (3.49 <sup>cd</sup> )
August	1.00 (1.23)	2.69 (1.79)	9.51 (3.16)	11.15 (3.41)	14.85 (3.92)	18.90 (4.41)	22.86 (4.83)	19.08 (4.43)	12.59 (3.62)	16.80 (4.16)	12.15 (3.56)	10.63 (3.34)	12.68 (3.49 <sup>cd</sup> )
Mean	2.48 (1.64 <sup>d</sup> )	10.56 (3.08 <sup>e</sup> )	15.27 (3.78 <sup>d</sup> )	19.56 (4.36 <sup>abc</sup> )	21.81 (4.60 <sup>ab</sup> )	22.38 (4.71 <sup>a</sup> )	21.24 (4.63 <sup>a</sup> )	19.02 (4.37 <sup>abc</sup> )	17.01 (4.13 <sup>bcd</sup> )	16.36 (4.05 <sup>cd</sup> )	14.07 (3.77 <sup>d</sup> )	10.16 (3.17 <sup>e</sup> )	

Factors Months (M)

Time (T)

M x T

SEm±

CD at 5%

0.453

0.496

Means in the columns/ rows with same alphabet do not differ significantly by DMRT at 5%

Figures in the parenthesis are  $\sqrt{(x + 0.5)}$  transformed values

Table 4. Outgoing foragers during north-east monsoon season

Months	Mean number of bees/5 minutes/colony - Hours →											Mean
	0600	0700	0800	0900	1000	1100	1200	1300	1400	1500	1600	1700
November	3.27 (1.94)	8.42 (2.99)	33.16 (5.80)	29.68 (5.49)	14.96 (3.93)	20.23 (4.55)	16.68 (4.15)	11.72 (3.49)	11.87 (3.51)	14.56 (3.88)	8.23 (2.95)	4.72 (2.28)
December	1.00 (1.23)	3.20 (1.92)	28.13 (5.35)	23.15 (4.86)	13.96 (3.8)	22.39 (4.78)	12.95 (3.67)	9.23 (3.12)	11.20 (3.42)	12.40 (3.59)	5.98 (2.55)	2.73 (1.79)
Mean	2.14 (1.58 <sup>d</sup> )	5.81 (2.45 <sup>d</sup> )	30.65 (5.58 <sup>b</sup> )	26.42 (5.18 <sup>a</sup> )	14.46 (3.87 <sup>c</sup> )	21.31 (4.67 <sup>d</sup> )	14.82 (3.91 <sup>d</sup> )	10.48 (3.31 <sup>d</sup> )	11.53 (3.47 <sup>c</sup> )	13.48 (3.74 <sup>d</sup> )	7.11 (2.75 <sup>d</sup> )	3.72 (2.04 <sup>d</sup> )

Factors  
Months (M)  
Time (T)  
M x T

SEm±  
0.044  
0.109  
0.154

CD at 5%  
0.118  
0.289  
0.435

Means in the columns/ rows with same alphabet do not differ significantly by DMRT at 5%

Figures in the parenthesis are  $\sqrt{(x + 0.5)}$  transformed values

Table 5. Outgoing foragers during dry season

Months	Mean number of bees/5 minutes/colony - Hours →											Mean
	0600	0700	0800	0900	1000	1100	1200	1300	1400	1500	1600	1700
January	1.00 (1.23)	1.42 (1.39)	11.79 (3.51)	27.38 (5.28)	24.16 (4.97)	11.30 (3.44)	19.42 (4.46)	15.90 (4.05)	8.06 (2.93)	7.87 (2.89)	7.83 (2.87)	5.77 (2.50)
February	1.60 (1.45)	18.41 (4.35)	56.84 (7.57)	48.51 (7.00)	31.21 (5.63)	30.64 (5.58)	27.48 (5.29)	16.52 (4.13)	17.71 (4.27)	13.83 (3.79)	18.01 (4.30)	7.48 (2.83)
March	1.00 (1.23)	30.45 (5.56)	34.97 (5.96)	48.93 (7.03)	56.08 (7.52)	41.56 (6.49)	31.96 (5.69)	22.09 (4.75)	27.06 (5.25)	24.27 (4.98)	15.65 (4.02)	11.66 (3.49)
April	10.73 (3.35)	32.89 (5.78)	49.84 (7.09)	55.03 (7.45)	45.77 (6.80)	38.40 (6.24)	34.61 (5.93)	33.61 (5.84)	32.93 (5.78)	26.86 (5.23)	27.71 (5.31)	18.90 (4.41)
May	9.75 (3.20)	25.32 (5.08)	29.12 (5.44)	39.99 (6.36)	29.08 (5.44)	30.24 (5.54)	24.30 (4.98)	14.48 (3.87)	25.10 (5.06)	19.53 (4.48)	14.77 (3.91)	13.3 (2.72)
Mean	4.82 (2.09 <sup>a</sup> )	21.69 (4.43 <sup>e</sup> )	36.51 (5.91 <sup>abc</sup> )	43.97 (6.62 <sup>a</sup> )	37.26 (6.07 <sup>ab</sup> )	30.43 (5.45 <sup>bc</sup> )	27.55 (5.27 <sup>cd</sup> )	20.52 (4.52 <sup>de</sup> )	22.17 (4.65 <sup>de</sup> )	18.47 (4.27 <sup>c</sup> )	16.79 (4.08 <sup>cd</sup> )	11.42 (3.38 <sup>f</sup> )

Factors  
Months (M)  
Time (T)  
M x T

SEm±  
0.177  
0.275

CD at 5%  
0.508  
0.788  
0.615

Means in the columns/ rows with same alphabet do not differ significantly by DMRT at 5%

Figures in the parenthesis are  $\sqrt{(x + 0.5)}$  transformed values

Table 6. Outgoing foragers during south-west monsoon season

Months	Mean number of bees/5 minutes/colony - Hours →												Mean
	0600	0700	0800	0900	1000	1100	1200	1300	1400	1500	1600	1700	
June	7.15 (2.77)	10.92 (3.38)	21.71 (4.71)	24.47 (4.99)	20.63 (4.59)	19.19 (4.44)	15.01 (3.94)	15.82 (4.04)	12.57 (3.62)	18.15 (4.32)	16.15 (4.1)	4.56 (2.25)	15.53 (3.92 <sup>a</sup> )
July	4.52 (2.24)	4.60 (2.26)	13.92 (3.79)	20.69 (4.60)	23.86 (4.94)	28.38 (5.37)	21.34 (4.67)	14.86 (3.92)	12.35 (3.59)	15.04 (3.94)	9.03 (3.1)	5.83 (2.51)	14.53 (3.74 <sup>a</sup> )
August	1.00 (1.23)	3.42 (1.98)	11.83 (3.51)	17.47 (4.24)	27.94 (5.33)	23.65 (4.91)	30.57 (5.57)	21.07 (4.64)	11.46 (3.46)	16.54 (4.13)	9.31 (3.13)	6.53 (2.65)	15.07 (3.73 <sup>a</sup> )
Mean	4.22 (2.08 <sup>d</sup> )	6.31 (2.54 <sup>d bed</sup> )	15.82 (4.00 <sup>ab</sup> )	20.88 (4.61 <sup>a</sup> )	24.15 (4.95 <sup>abc</sup> )	23.74 (4.91 <sup>bed</sup> )	22.30 (4.73 <sup>abc</sup> )	17.25 (4.20 <sup>bc</sup> )	12.13 (3.55 <sup>cd</sup> )	16.57 (4.13 <sup>abc</sup> )	11.50 (3.43 <sup>abc</sup> )	5.64 (2.47 <sup>cd</sup> )	

Factors  
Months (M)  
Time (T)  
M x T

SEm±  
0.174  
0.347  
0.602

CD at 5%  
0.209  
0.419

Means in the columns/ rows with same alphabet do not differ significantly by DMRT at 5%

Figures in the parenthesis are  $\sqrt{(x + 0.5)}$  transformed values

Table 7. Incoming foragers with pollen during north-east monsoon season

Months	Mean number of bees/5 minutes/colony - Hours →												Mean
	0600	0700	0800	0900	1000	1100	1200	1300	1400	1500	1600	1700	
November	1.00 (1.23)	2.39 (1.7)	15.72 (4.03)	18.74 (4.39)	13.56 (3.75)	7.15 (2.77)	2.90 (1.85)	2.70 (1.79)	1.47 (1.40)	2.18 (1.64)	1.00 (1.23)	1.00 (1.23)	5.82 (2.24 <sup>a</sup> )
December	1.00 (1.23)	1.47 (1.31)	13.73 (3.77)	13.33 (3.72)	9.42 (3.15)	5.98 (2.55)	3.97 (2.12)	3.92 (2.10)	2.24 (1.65)	2.09 (1.61)	1.47 (1.40)	1.23 (1.31)	4.99 (2.1 <sup>a</sup> )
Mean	1.00 (1.22 <sup>g</sup> )	1.93 (1.50 <sup>cd</sup> )	14.72 (3.9 <sup>u</sup> )	16.03 (4.05 <sup>a</sup> )	11.49 (3.45 <sup>b</sup> )	6.56 (2.65 <sup>c</sup> )	3.44 (1.98 <sup>d</sup> )	3.31 (1.94 <sup>d</sup> )	1.85 (1.52 <sup>ef</sup> )	2.14 (1.62 <sup>e</sup> )	1.23 (1.31 <sup>fg</sup> )	1.11 (1.26 <sup>g</sup> )	

Factors  
Months (M)  
Time (T)  
M x T

SEm±  
0.039  
0.096  
0.136

CD at 5%  
0.133  
0.325  
0.385

Means in the columns/ rows with same alphabet do not differ significantly by DMRT at 5%

Figures in the parenthesis are  $\sqrt{(x + 0.5)}$  transformed values



Table 8. Incoming foragers with pollen during dry season

Months	Mean number of bees/5 minutes/colony - Hours →												Mean
	0600	0700	0800	0900	1000	1100	1200	1300	1400	1500	1600	1700	
January	1.00 (1.23)	1.00 (1.23)	4.42 (2.22)	14.84 (3.92)	10.82 (3.37)	4.55 (2.25)	3.39 (1.97)	3.08 (1.89)	2.39 (1.7)	3.10 (1.9)	2.83 (1.83)	2.35 (1.68)	4.48 (2.09 <sup>b</sup> )
February	1.00 (1.23)	3.42 (1.98)	19.30 (4.45)	23.76 (4.93)	11.90 (3.52)	13.07 (3.68)	4.89 (2.32)	6.22 (2.59)	4.66 (2.27)	3.57 (2.02)	1.87 (1.54)	1.42 (1.39)	7.92 (2.65 <sup>a</sup> )
March	1.00 (1.23)	3.46 (1.99)	9.48 (3.16)	17.70 (4.27)	23.94 (4.94)	12.68 (3.63)	8.47 (2.99)	4.85 (2.31)	2.77 (1.81)	2.69 (1.79)	2.39 (1.7)	2.09 (1.61)	2.09 (2.61 <sup>a</sup> )
April	1.47 (1.4)	3.62 (2.03)	18.10 (4.31)	25.26 (5.08)	21.43 (4.68)	10.87 (3.37)	6.10 (2.57)	6.22 (2.59)	4.47 (2.23)	3.21 (1.93)	2.51 (1.73)	1.60 (1.45)	8.74 (2.78 <sup>a</sup> )
May	1.00 (1.23)	4.81 (2.3)	7.54 (2.84)	5.15 (2.38)	4.49 (2.23)	4.08 (2.14)	2.90 (1.85)	3.08 (1.89)	2.39 (1.7)	1.68 (1.48)	1.95 (1.57)	1.00 (1.23)	3.34 (1.90 <sup>b</sup> )
Mean	1.09 (1.26 <sup>f</sup> )	3.26 (1.90 <sup>de</sup> )	11.77 (3.39 <sup>bc</sup> )	17.34 (4.11 <sup>a</sup> )	14.52 (3.75 <sup>ab</sup> )	9.05 (3.01 <sup>c</sup> )	5.15 (2.34 <sup>d</sup> )	4.69 (2.25 <sup>d</sup> )	3.34 (1.94 <sup>de</sup> )	2.85 (1.82 <sup>de</sup> )	2.31 (1.67 <sup>ef</sup> )	1.69 (1.47 <sup>ef</sup> )	

Factors

Months (M)

Time (T)

M x T

SEm±

0.143

0.221

CD at 5%

0.430

0.667

0.495

Means in the columns/ rows with same alphabet do not differ significantly by DMRT at 5%

Figures in the parenthesis are  $\sqrt{(x + 0.5)}$  transformed values

Table 9. Incoming foragers with pollen during south-west monsoon season

Months	Mean number of bees/5 minutes/colony - Hours →												Mean
	0600	0700	0800	0900	1000	1100	1200	1300	1400	1500	1600	1700	
June	1.47 (1.4)	3.00 (1.87)	4.85 (2.31)	3.55 (2.01)	3.49 (1.99)	2.99 (1.87)	1.47 (1.4)	2.09 (1.61)	1.68 (1.48)	2.49 (1.73)	2.15 (1.63)	1.00 (1.23)	2.52(1.71 <sup>b</sup> )
July	1.00 (1.23)	1.68 (1.48)	2.84 (1.83)	6.19 (2.59)	7.62 (2.85)	8.87 (3.06)	7.78 (2.88)	3.98 (2.12)	2.77 (1.81)	2.49 (1.73)	1.77 (1.51)	1.47 (1.4)	4.04 (2.04 <sup>a</sup> )
August	1.00 (1.23)	1.42 (1.39)	7.50 (2.83)	8.04 (2.92)	9.04 (3.09)	12.71 (3.63)	9.59 (3.18)	8.66 (3.03)	2.21 (1.65)	3.90 (2.1)	2.15 (1.63)	1.23 (1.31)	5.62 (2.33 <sup>a</sup> )
Mean	1.16 (1.28 <sup>c</sup> )	2.03 (1.58 <sup>de</sup> )	5.06 (2.32 <sup>abc</sup> )	5.93 (2.50 <sup>ab</sup> )	6.71 (2.64 <sup>ab</sup> )	8.19 (2.85 <sup>a</sup> )	6.28 (2.48 <sup>ab</sup> )	4.91 (2.25 <sup>bc</sup> )	2.22 (1.64 <sup>de</sup> )	2.96 (1.85 <sup>cd</sup> )	2.02 (1.59 <sup>de</sup> )	1.23 (1.31 <sup>de</sup> )	

Factors  
Months (M)  
Time (T)  
M x T

SEM±  
0.107  
0.214  
0.37

CD at 5%  
0.333  
0.667

Means in the columns/ rows with same alphabet do not differ significantly by DMRT at 5%

Figures in the parenthesis are  $\sqrt{(x + 0.5)}$  transformed values

Table 10. Incoming foragers without pollen during north-east monsoon season

	Mean number of bees/5 minutes/colony - Hours →												Mean
	0600	0700	0800	0900	1000	1100	1200	1300	1400	1500	1600	1700	
Months	0600	0700	0800	0900	1000	1100	1200	1300	1400	1500	1600	1700	Mean
November	1.68 (1.48)	6.75 (2.69)	5.67 (2.48)	8.66 (3.03)	11.67 (3.49)	15.43 (3.99)	16.96 (4.18)	13.67 (3.76)	15.68 (4.02)	12.19 (3.56)	9.47 (3.16)	6.99 (2.74)	10.40 (3.21 <sup>a</sup> )
December	1.00 (1.23)	2.48 (1.73)	5.56 (2.46)	8.23 (2.96)	9.39 (3.15)	17.22 (4.21)	17.73 (4.27)	11.71 (3.49)	10.97 (3.39)	10.17 (3.27)	12.21 (3.57)	5.98 (2.55)	9.39 (3.02 <sup>b</sup> )
Mean	1.34 (1.35 <sup>e</sup> )	4.61 (2.21 <sup>f</sup> )	5.61 (2.47 <sup>ef</sup> )	8.44 (2.99 <sup>d</sup> )	10.53 (3.31 <sup>c</sup> )	16.32 (4.1 <sup>a</sup> )	17.34 (4.22 <sup>a</sup> )	12.69 (3.63 <sup>bc</sup> )	13.33 (3.70 <sup>b</sup> )	11.18 (3.41 <sup>bc</sup> )	10.84 (3.36 <sup>c</sup> )	6.48 (2.64 <sup>c</sup> )	

Factors  
Months (M)  
Time (T)  
M x T

SEM±  
0.046  
0.112  
0.158

CD at 5%  
0.138  
0.34  
0.446

Means in the columns/ rows with same alphabet do not differ significantly by DMRT at 5%

Figures in the parenthesis are  $\sqrt{(x + 0.5)}$  transformed values

Table 11. Incoming foragers without pollen during dry season

Months	Mean number of bees/5 minutes/colony - Hours →												Mean
	0600	0700	0800	0900	1000	1100	1200	1300	1400	1500	1600	1700	
January	1.00 (1.23)	1.00 (1.23)	5.41 (2.43)	15.42 (3.99)	13.47 (3.74)	11.51 (3.47)	18.44 (4.35)	10.90 (3.38)	9.04 (3.09)	9.75 (3.2)	7.36 (2.8)	5.28 (2.40)	9.05 (2.94 <sup>c</sup> )
February	1.42 (1.39)	14.36 (3.86)	31.83 (5.69)	28.35 (5.37)	22.86 (4.83)	27.91 (5.33)	21.36 (4.68)	19.73 (4.49)	17.03 (4.19)	18.28 (4.33)	19.28 (4.45)	9.35 (3.14)	19.31 (4.31 <sup>b</sup> )
March	1.00 (1.23)	25.28 (5.08)	23.06 (4.85)	25.83 (5.13)	38.56 (6.25)	30.07 (5.53)	23.69 (4.92)	21.93 (4.74)	24.55 (5.01)	22.63 (4.81)	17.55 (4.25)	17.36 (4.23)	22.62 (4.66 <sup>b</sup> )
April	7.11 (2.76)	20.98 (4.64)	27.06 (5.25)	32.64 (5.76)	41.28 (6.46)	35.52 (6.00)	30.55 (5.57)	30.40 (5.56)	24.90 (5.04)	26.22 (5.17)	22.78 (4.83)	23.83 (4.93)	26.94 (5.16 <sup>c</sup> )
May	4.45 (2.23)	17.20 (4.21)	22.33 (4.78)	30.83 (5.59)	26.34 (5.18)	31.94 (5.69)	29.06 (5.44)	25.56 (5.11)	23.03 (4.85)	18.93 (4.41)	15.52 (4.00)	9.54 (3.17)	21.23 (4.55 <sup>b</sup> )
Mean	3.00 (1.76 <sup>b</sup> )	15.76 (3.8 <sup>de</sup> )	21.94 (4.6 <sup>abc</sup> )	26.61 (5.16 <sup>a</sup> )	28.50 (5.29 <sup>a</sup> )	27.39 (5.20 <sup>a</sup> )	24.62 (4.99 <sup>ab</sup> )	21.70 (4.65 <sup>abc</sup> )	19.71 (4.43 <sup>bcd</sup> )	19.16 (4.38 <sup>bcd</sup> )	16.50 (4.06 <sup>cde</sup> )	13.07 (3.57 <sup>c</sup> )	

CD at 5%

0.478

0.741

SEm±

0.165

0.256

0.572

Factors

Months (M)

Time (T)

M x T

Means in the columns/ rows with same alphabet do not differ significantly by DMRT at 5%

Figures in the parenthesis are  $\sqrt{(x + 0.5)}$  transformed values

Table 12. Incoming foragers without pollen during south-west monsoon season

Months	Mean number of bees/5 minutes/colony - Hours →												Mean
	0600	0700	0800	0900	1000	1100	1200	1300	1400	1500	1600	1700	
June	4.49 (2.23)	9.89 (3.22)	14.90 (3.92)	21.19 (4.66)	19.13 (4.43)	14.08 (3.82)	11.89 (3.52)	17.58 (4.25)	15.76 (4.03)	17.28 (4.22)	15.27 (3.97)	6.30 (2.61)	13.98 (3.74 <sup>a</sup> )
July	1.68 (1.48)	5.01 (2.35)	7.33 (2.79)	13.27 (3.71)	20.55 (4.59)	21.25 (4.66)	19.89 (4.52)	19.69 (4.49)	16.58 (4.13)	11.31 (3.44)	9.12 (3.1)	6.38 (2.62)	12.67 (3.49 <sup>a</sup> )
August	1.00 (1.23)	2.69 (1.79)	9.51 (3.16)	11.15 (3.41)	14.85 (3.92)	18.90 (4.41)	18.90 (4.83)	19.08 (4.43)	12.59 (3.62)	16.80 (4.16)	12.15 (3.56)	10.63 (3.34)	12.68 (3.49 <sup>a</sup> )
Mean	2.39 (1.65 <sup>c</sup> )	5.86 (2.45 <sup>de</sup> )	10.58 (3.30 <sup>bcd</sup> )	15.20 (3.93 <sup>ab</sup> )	18.18 (4.31 <sup>a</sup> )	18.08 (4.29 <sup>a</sup> )	18.21 (4.29 <sup>a</sup> )	18.78 (4.39 <sup>a</sup> )	14.98 (3.93 <sup>ab</sup> )	15.13 (3.94 <sup>ab</sup> )	12.18 (3.54 <sup>abc</sup> )	7.77 (2.85 <sup>cd</sup> )	

CD at 5%

0.514

1.028

SEm±

0.172

0.345

0.597

Factors

Months (M)

Time (T)

M x T

Means in the columns/ rows with same alphabet do not differ significantly by DMRT at 5%

Figures in the parenthesis are  $\sqrt{(x + 0.5)}$  transformed values

least during early morning hours and gradually increased to attain the first peak during 0900-1000 h and a slight second peak at 1500 h (Table 2). After the second peak, the activity reduced until 1800 h. The number of incoming bees without pollen loads was recorded to be least during early morning hours 0600-0700 h where after it gradually increased to reach a peak during 1100-1200 h (Table 3). The activity gradually decreased for the rest of the day.

**Monthly variation in foraging pattern:** The maximum activity of outgoing foragers around the year was observed during April (33.94 bees/5 min) followed by March (28.81 bees/5 min) and the least activity was during January (11.82 bees/5 min). The highest activity was during hotter months and the lowest activity during the winter months. The month of April exhibited the highest activity of incoming foragers with pollen (8.74 bees/5 min) followed by February (7.92 bees/5 min). The month of June (2.52 bees/5 min) and May (3.34 bees/5 min) observed the least activity. The activity was highest in the dry season while lowest in the south-west monsoon season. The greatest activity in the incoming of non-pollen foragers was in the month of April (26.94 bees/5 min) followed by March (22.62 bees/5 min) while the least was during January (9.05 bees/5 min). The maximum activity was during hotter months of the dry season and the least during the winter period.

**Foraging activity of outgoing foragers:** The outgoing foragers were active throughout the months in the north-east monsoon season (Table 4). The activity was on par for both the months in the season. Peak activity in the season occurred during the initial part of late morning hours from 0800-0900 h (30.65 bees/ 5 min) which then declined gradually to reach a second peak at 1100-1200 h (21.31 bees/ 5 min) which again decreased to reach the third peak at 1500 hrs (13.48 bees/ 5 min). The least activity was observed during 0600-0700 h (2.14 bees/ 5 min) and 1700-1800 h (3.72 bees/ 5 min). The outgoing foragers were most active in the dry season and differed between months of the season as well as hours of the day (Table 5). The month of April significantly differed from every other month (33.94 bees/ 5 min) which marked the

highest activity of the season. The months of February (24.02 bees/ 5 min), March (28.81 bees/ 5 min) and May (22.92 bees/ 5 min) were on par whereas the lowest was observed during the cold month of January (11.82 bees/ 5 min). The first peak of the day was observed during 0900-1000 hrs (43.97 bees/ 5 min) after which it declined and reached a second peak at 1400 hrs (22.17 bees/ 5 min). The lowest activity of the day was recorded in the early morning 0600-0700 h. The outgoing foragers during the south-west monsoon season were moderately active and statistically on par in all three months with a slighter higher number of foragers during June month (15.92 bees/ 5 min). The hour with the least activity was from 0600-0700 (4.22 bees/ 5 min) which gradually increased to the first peak of activity at 1000 h (24.15 bees/ 5 min). The activity declined afterwards to certain hours after which it attained a second peak at 1500 h (16.57 bees/ 5 min) which again declined until evening (Table 6).

**Foraging activity of incoming foragers with pollen:** The incoming foragers with pollen loads were also on par for both the months with a slightly higher value during November (5.82 bees/ 5 min). The least activity was during early morning hours from 0600-0700 h, and then increased to reach the first peak of activity 0900-1000 h (16.03 bees/ 5 min). After the first peak, the activity declined for a few hours and again attained a second peak at 1500 hrs (2.14 bees/ 5 min) after which it decreased until 1800 h (Table 7).

The incoming foragers with pollen during the dry season (Table 8) was recorded the highest in April (8.74 bees/ 5 min) which was on par with the months February (7.92 bees/ 5 min) and March (7.63 bees/ 5 min). The month of May had the least activity (3.34 bees/ 5 min) which was on par with January (4.48 bees/ 5 min). The activity was least during morning 0600-0700 h which then increased to a peak at 0900 hrs (17.34 bees/ 5 min). After the peak, the activity decreased gradually to a lower value until 1800 h. The incoming foragers with pollen loads were lowest in the month of June (2.52 bees/ 5 min). The month of August (5.62 bees/ 5 min) and July (4.04 bees/ 5 min) was on par (Table 9). The maximum activity of incoming pollen foragers



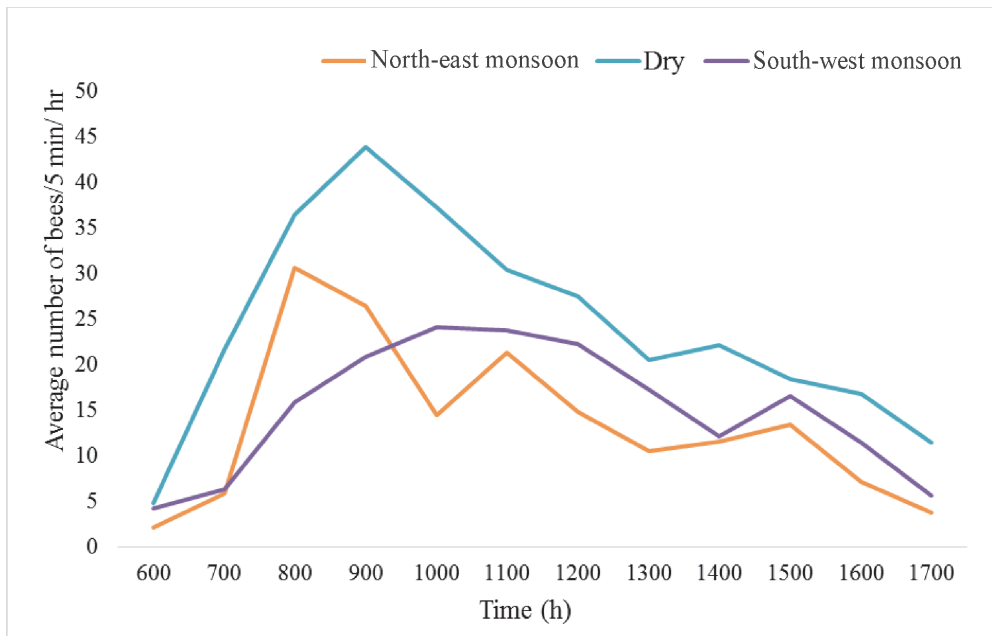


Fig 1. The foraging activity of outgoing foragers during north-east monsoon (November- December), dry (January- May) and south-west monsoon (June- August) season

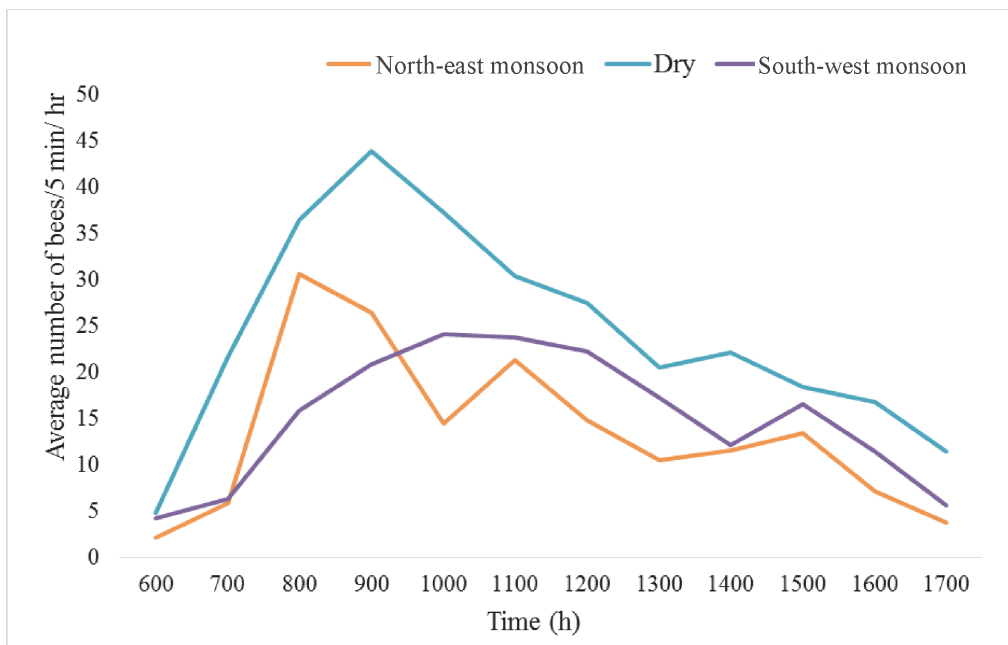


Fig 2. The foraging activity of incoming foragers with pollen during north-east monsoon (November- December), dry (January- May) and south-west monsoon (June- August) season

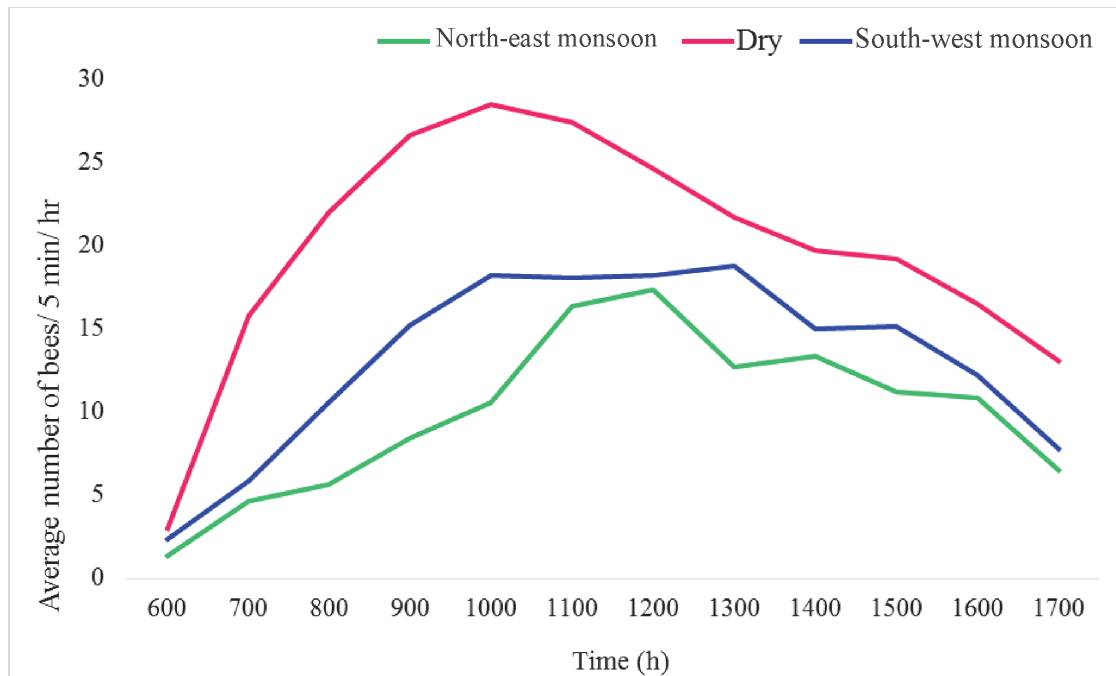


Fig 3. The foraging activity of incoming foragers without pollen during north-east monsoon (November- December), dry (January- May) and south-west monsoon (June- August) season

was from 1100-1200 h (8.19 bees/ 5 min), then it decreased for few hours and again increased into a slight peak during 1500-1600 hrs (2.96 bees/ 5 min). The activity was lowest during 0600-0700 h and 1700-1800 h (1.16 bees/ 5 min).

**Foraging activity of incoming foragers without pollen:** The incoming foragers without pollen loads were significantly different between months with a higher number in November (10.40 bees/ 5 min). The lowest number of bees was observed from 0600-0700 h (1.34 bees/ 5 min) in the early morning (Table 10), then increased gradually to a peak at 1200 h (17.34 bees/ 5 min), again to fall gradually until evening hour 1800. The incoming foragers without pollen loads were the highest during April (26.94 bees/ 5 min) which significantly differed from every other month (Table 11). The months of February (19.31 bees/ 5 min), March (22.62 bees/ 5 min) and May (21.23 bees/ 5 min) were on par while January with the lowest value (9.05 bees/ 5 min) differed significantly. The peak activity of the

day was during 1000-1100 h (28.50 bees/ 5 min) and the lowest value of the day (3 bees/ 5 min) during 0600-0700 h. After the peak, the activity gradually decreased for the rest of the day until 1800 h. The incoming foragers without pollen loads did not differ significantly between the months which had their highest number of bees during June (13.98 bees/ 5 min). The activity of incoming non-pollen foragers was lowest during early morning 0600-0700 h (2.39 bees/ 5 min) which increased gradually to reach the first peak at 1000 hrs (18.18 bees/ 5 min) and a second peak during 1300-1400 h (18.78 bees/ 5 min) which again reached a third slight peak at 1500 h (15.13 bees/ 5 min). The activity then decreased gradually until 1800 h (Table 12).

## DISCUSSION

The foraging activity of stingless bees differed between the three seasons due to the fluctuations in weather conditions and time-based disparity in resource availability.

**Diurnal variation in foraging activity:** The foraging activity started as early as 0600 hrs during April and May which are the months of the dry season with high average temperature. This is rather early to the formerly reported starting time by Devanesan *et al.* (2002) which was 0700 h in Kerala. Meanwhile, foraging started later between 0645 and 0700 h during December and January with cool, chilly or foggy mornings. Similar slight late initiation of activity during colder months December and January was reported by Roopa (2002). During the rainy months of June, July and August too, the activity started late around 0610 h. The foraging activity continued up to 1840 h during the dry hot season, though in other seasons, the activity ended in advance.

The highest foraging activity of outgoing and incoming pollen foragers was between 0800-1100 h in the late morning period whereas incoming foragers without pollen were most active during 1000-1300 h. The study on foraging behaviour conducted by Biesmeijer *et al.* (1992) showed that 75 per cent of pollen collection took place from 0800-1000 h which substantiate the present findings. The higher foraging activity, especially pollen gathering in the late morning hours (0900-1100 h), might be due to the need to fulfil their energy prerequisite, ideal accessibility of floral resources and favourable climatic conditions. This has also been documented by Azmi *et al.* (2015) for *Lepidotrigona terminata*. The foraging for nectar which was the function of non-pollen foragers seemed to increase during noon hours (1200-1300 h) due to the more sugar concentration in nectar. This is in line with the findings of Kajobe (2007) indicating that the sugar concentration of bee collected nectar increased from morning hours till 1300-1400 h, which might be due to the higher solar radiation causing evaporation and resulting in more concentrated nectar in flowers (Roubik and Buchmann, 1984; Roubik, 1989). In the afternoon hours (1300-1500) less activity has been observed in the hives, probably to conserve energy. The lowest activity observed during early morning hour (0600-0700) and late evening hour (1700-1800) might be attributed to non-favourable temperature,

diminished sunlight intensity and also less availability of pollen and nectar.

**Monthly variation in foraging activity:** The greatest average activity evident during the dry season which included the hotter months of the year, March, April and May could be possibly due to the favourable weather conditions such as optimum temperature and low relative humidity. This was confirmed by the findings of Nunes-Silva *et al.* (2010) who stated that pollen and nectar foragers are positively correlated to temperature and negatively to humidity. Similar findings were also reported by Bharath *et al.* (2020) who stated that the highest activity was during March and reduced to the end of May. The higher activity in the month of July and August during the south-west monsoon could be attributed to the influence of rainfall on the blooming pattern which was also observed by Devanesan *et al.* (2009) and Aleixo *et al.* (2017). The overall activity was seen lowest during two months viz., January and December which were the coldest months, for which the low temperature can be held accountable. Pedro and Camargo (1991) and Managanvi *et al.* (2012) reported similar to findings.

**Foraging activity of outgoing foragers:** The activity of outgoing foragers in the north-east monsoon season attained three distinct peaks from 0800-0900, 1100-1200 and 1500-1600 h whereas south-west monsoon and dry seasons were observed to have two peaks (Fig. 1), one in the morning between 0900-1100 h and another one in the afternoon during 1500-1600 h. The studies by Roopa (2002) showed that during monsoon season, two distinct peaks of outgoing foragers were observed between 1200-1300 and 1600-1700 h. Similarly, Ghazi *et al.* (2014) noted that the effective time for foragers to go out was early in the morning between 0800 to 1100 hrs and the peak of activity was 1000 and 1200 h.

**Foraging activity of incoming pollen foragers:** The activity of incoming foragers with pollen was highest in between 0900 and 1000 h in the north-east monsoon, the dry season and 1100-1200 h during the south-west monsoon season. The second

peak was later in the afternoon during 1500-1600 h in all three seasons (Fig. 2). This is in accordance with the study conducted by Layek and Karmakar (2018) in West Bengal where they obtained two distinct peaks occurring between 0900-1100 and 1500-1600 h and also Devanesan *et al.* (2002) who reported two distinct peaks at 1200 and 1500 h respectively.

**Foraging activity of incoming forgers without pollen:** The activity of incoming non-pollen foragers showed only one distinct peak between 1000-1200 h except in south-west monsoon which had three peaks viz., 1000-1100 h (first peak), 1300-1400 h (slight second peak) and 1500-1600 h (third peak) (Fig. 3). Foraging activity that had a single peak was also obtained by Danaraddi *et al.* (2011) in Karnataka. The variations in the activity might be due to difference in colony health, climatic aspects and flora of the area. The study provides information on the foraging activity pattern of *T. travancorica* which is higher during morning hours from 0800-1200 h and late afternoon 1500-1600 h. It can be concluded that the foraging activity is highest during the summer months and lowest during the winter months. The change in the pattern of foraging behaviour with seasons suggests a strong influence of weather and flora on the bee colony.

## ACKNOWLEDGEMENT

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## New records of Chalcididae (Hymenoptera: Chalcidoidea) from Yemen

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**ABSTRACT:** The present study deals with the new country records and generic records of two genera of family Chalcididae, viz., *Epitranus clavatus* (Fabricius) and *Hockeria tamaricis* Bouèek. Both recorded species are detailed with diagnosis, host specificity and geographical distribution.

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**KEY WORDS:** Chalcididae, *Hockeria*, *Epitranus*, new records, Yemen

Chalcididae (Hymenoptera: Chalcidoidea) is a family of small wasps parasitizing several insect pests of agricultural and medical importance. Despite their high economic importance, they are poorly known from Middle Eastern region of the world. Boucek (1956) reported the occurrence of the family Chalcididae and recorded *Dirhinus wohlfahrtiae* Ferrière from Yemen. Since then no any chalcidid species recorded from Yemen. However, chalcidid fauna were reported from other countries of the Middle East. Some of the recent works that deserve attention are as follows. Delvare (2017) indicated that at least 74 species of Chalcididae are present in UAE, representing about half of the described species in the Palaearctic region. Gul *et al.* (2018) altogether reported and described seven species of *Dirhinus* Dalman from different regions of Saudi Arabia. Moravvej *et al.* (2018) reported *Epitranus clavatus* from Iran for

the first time. Gul *et al.* (2020) and Gadallah *et al.* (2020) reported the genus *Phasgonophora* Westwood and *Epitranus* Walker for the first time from the Kingdom of Saudi Arabia with the description of five and seven species respectively.

Here we report two genera *Epitranus* Walker (1834) and *Hockeria* Walker (1834) with species: *E. clavatus* (Fabricius) and *H. tamaricis* Bouèek for the first time from Yemen. These belong to subfamilies Epitraninae Burks and Haltichellinae Ashmead respectively.

Specimens were collected through sweep net on grasses by one of the authors (FSKA). The collected material were dried and mounted on small rectangular cards (Qamar, 2017). The specimens were examined using Nikon SMZ25 stereomicroscope and photographed later. The photographs were retouched using Adobe

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Photoshop® CS3. Identified specimens are deposited in the Insect collections, Department of Zoology, Aligarh Muslim University, Aligarh, India (ZDAMU).

### List of Chalcididae from Yemen

#### I. Subfamily: Dirhininae

*Dirhinus wohlfahrtiae* Ferrière, 1935

#### II. Subfamily: Epitraninae (new record)

*Epitranus clavatus* (Fabricius, 1804)

#### III. Subfamily: Haltichellinae (new record)

*Hockeria tamaricis* Boucek, 1982b

#### *Epitranus clavata* (Fabricius, 1804) (Fig. 1)

*Chalcis clavata* Fabricius, 1804: 162; Bouček, 1982a: 594: lectotype designation.

**Material examined:** YEMEN: TAIZ: Wadi Dabab, 22.viii.2014, Coll. F.S.K. Amer; ♀ (on card), (ZDAMU).

**Diagnosis:** Female. Body largely testaceous brown (Fig. 1). Legs yellowish brown; tarsi yellow (Fig. 1a). Wings hyaline with minute pubescence. Head a little wider than its length and slightly over the width of thorax; scrobe striated, clypeus developed forward in conical shape (with teeth like structure) (Fig. 1c). Antenna not reaching the front ocellus and, with seven segmented funicle (Fig. 1b). Thorax with close pits, posterior side margins of pronotum slightly emarginated, scutellum somewhat convex anteriorly, apex rounded; propodeum with pre-current median area delimited by distinct submedian carinae, lateral teeth indistinct; wings hyaline with marginal vein faintly visible, pale yellow, a faint streak directed obliquely from stigma vein to basal region; ventral side of the hind coxa and outer disc of hind femora moderately pubescent; outer ventral margin of the hind femora with irregular nine teeth. Tooth of hind tibia crenulate formed by small five teeth, median tooth larger; tarsal sulcus not at all reaching tibial hump (Fig. 1d). Gaster with petiole slightly wider at base, with three carinae on dorsal side, gaster acuminate at apex (Figs. 1a, e).

**Hosts:** *Tinea antricola* Meyrick and *Crypsithyris* sp. (Bouček, 1982a).

**Distribution:** Yemen (new record). Worldwide (Bouček, 1982a; Noyes, 2021; (Moravvej *et al.*, 2018; Gadallah *et al.*, 2020).

*Hockeria tamaricis* Boucek, 1982b (Figs. 2, 3)

*Hockeria tamaricis* Bouček, 1982b: 49. Female, male. Holotype female, Israel, Michmoret (BMNH), not examined.

**Material examined:** YEMEN: TAIZ: Wadi Dabab, 22.viii.2014, Coll. F.S.K. Amer; ♀ (on card), (ZDAMU).

**Diagnosis:** Female. Body largely black; Pedicel, tegulae, and all legs except fore and mid tibiae as well as tarsi, reddish; gaster extensively to wholly red. Forewing with broad infuscation subdivided medially by two large hyaline spots (Fig. 3a). Head with markedly convex face; frons with numerous silver white hairs. Scrobal cavity shallow not reaching mid ocellus (Fig. 3c). Scape not reaching front ocellus; pedicel 1.8× as long as broad, subequal in length to second funicular segments. Thorax broadest across the pronotum, with punctuation, and almost globose-convex. Paraspidal furrows obliterated to indistinct thin lines. Popodeum with silvery white hairs. Hind femur about 2× as long as broad, with sharper tooth in the middle, hind tibia stout, distal tooth lobe-like, broad and low; comb starting on sharper tooth. Gaster ovate, pointed at apex, on a short petiole.

**Host:** Unknown for specimens from Yemen. For Saudi Arabia: lepidopterus gall maker on *Tamarix*. Elsewhere reared from pupae of *Amblypalpis olivierella* Ragonot (Bouček, 1982b).

**Distribution:** Yemen (new record). Israel, Pakistan, Saudi Arabia.

The study resulted the first record of two species as *Epitranus clavatus* (Fabricius) and *Hockeria tamaricis* Boucek from Yemen. Including these two species, the total number of known species in family chalcididae from Yemen now raised to three. The study also indicates the potential presence of

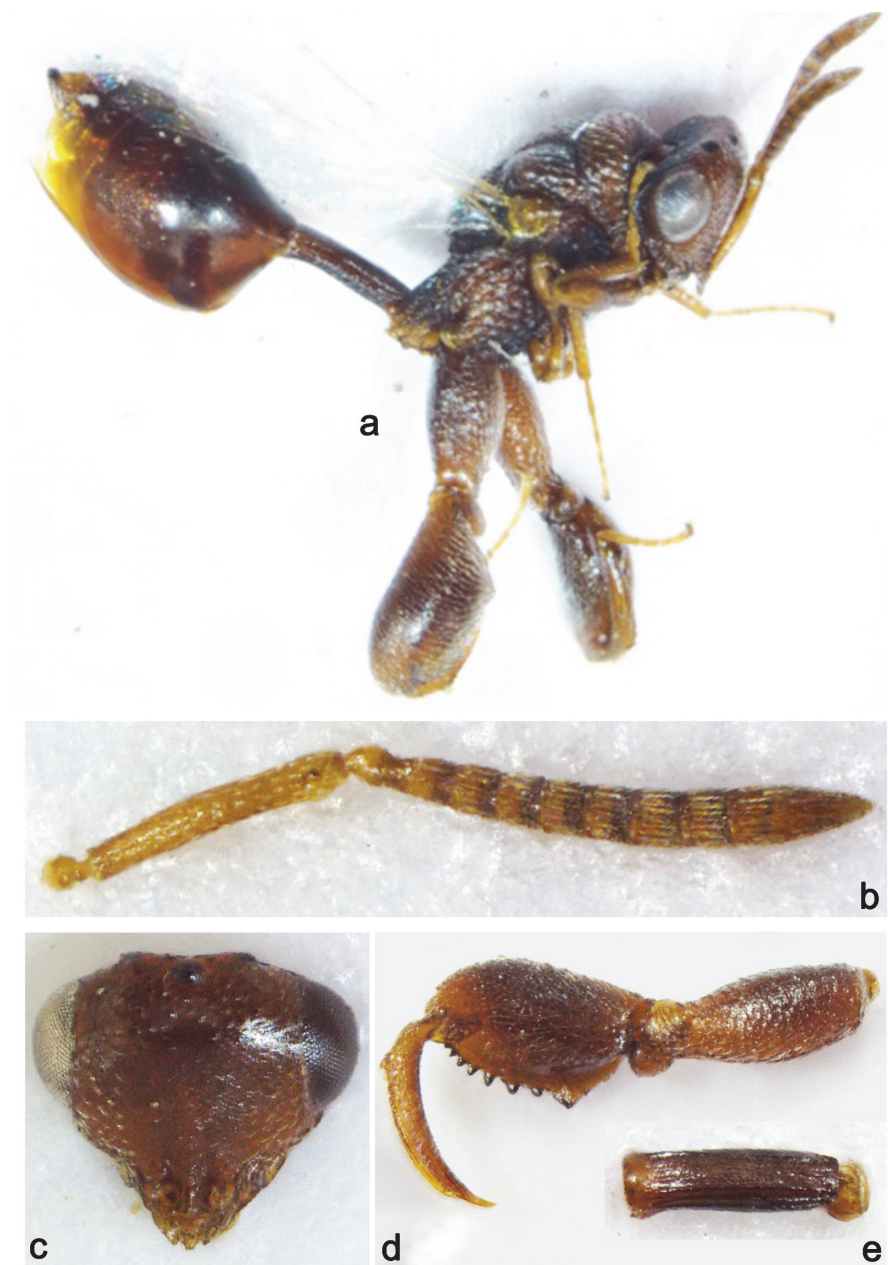


Fig. 1 *Epitranus clavatus* (F):  
a - Habitus, b - Antenna, C - Head, frontal, d - Hind leg, e - Petiole



Fig. 2 *Hockeria tamaricis* Bouck

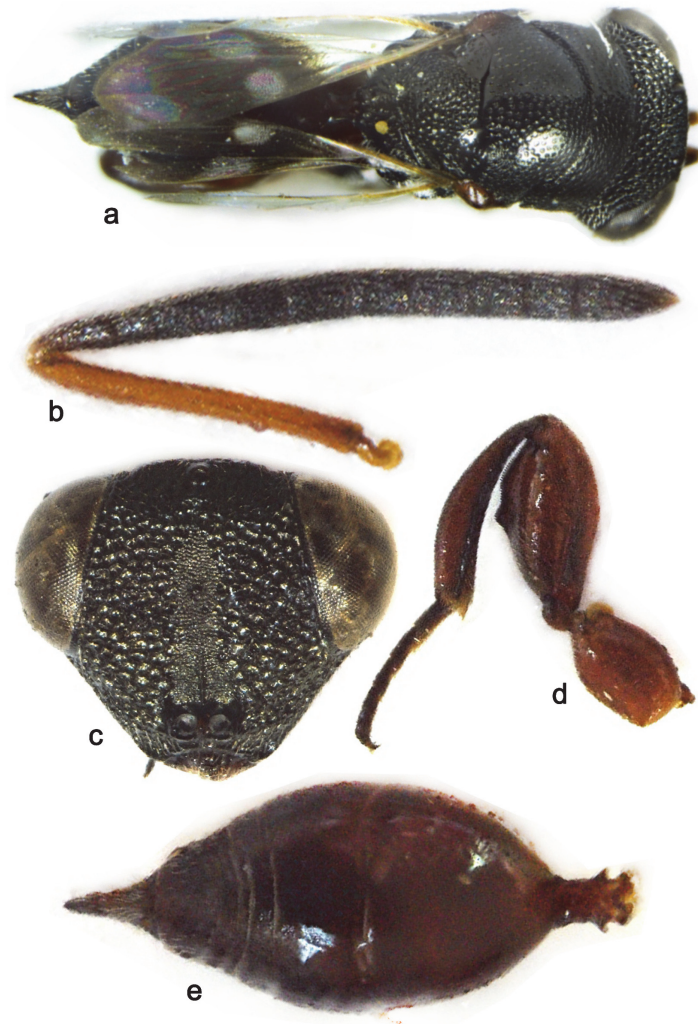


Fig. 3 *Hockeria tamaricis* Bouck:

a - Habitus, b - Antenna, c - Head, frontal, d - Hind leg, e - Gaster with petiole



unexplored chalcids in particular as well as other insects groups in general. Further extensive taxonomic studies along with biology and on host association are of considerable importance to understand the faunal diversity chalcid wasps in Yemen, which will provide the baseline future workers.

## ACKNOWLEDGEMENTS

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## First report of *Oxyophthalma engaea* (Wood-Mason, 1889) (Insecta: Mantodea: Eremiaphilidae) from Kerala, India

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**ABSTRACT:** The mantid species *Oxyophthalma engaea* (Wood-Mason, 1889) (Insecta: Mantodea: Eremiaphilidae) is reported for the first time from Kerala, India, and redescribed based on a single female specimen. © 2021 Association for Advancement of Entomology

**KEY WORDS:** Mantodea, Eremiaphilidae, *Oxyophthalma engaea*, First report, Kerala.

The genus *Oxyophthalma* Saussure belongs to the tribe Dysaulini in the subfamily Iridinae and family Eremiaphilidae; Dysaulini is currently known from India with three genera; *Dysaules* Stål, 1877; *Dysaulophthalma* Stiewe, 2009 and *Oxyophthalma* Saussure, 1861 (Schwarz and Roy, 2019). None of these genera were reported from the Kerala State till date. The genus *Oxyophthalma* is different from other genera in the group by the shape of eyes and lateral lobes; the lateral lobes are prolonged as a sharp tubercle and extend a little above the upper edge of the eyes. This genus is currently known by only two species, *Oxyophthalma engaea* (Wood-Mason, 1889) and *Oxyophthalma gracilis* Saussure, 1861, both are reported from India. The former species was reported from Andhra Pradesh, Tamil Nadu and Sri Lanka and the latter from Karnataka, Tamil Nadu and Sri Lanka (Mukherjee *et al.*, 2014). *O. engaea* differs from *O. gracilis* in having body covered with dense deep brown to black spots (Mukherjee *et al.*, 1995).

The specimens were collected from the Karyavattom Campus, Kerala University, Thiruvananthapuram, Kerala (Lat 8° 33' 43" N, Long 76° 53' 02" E, Alt 40m), by hand picking. The pinned specimens were examined using stereoscopic binocular microscope of model Leica M 205C and the photographs were taken with Leica DFC 500 camera and Canon EOS M50 camera. Images at varying depth were stacked using Leica Auto Montage Software V3.80. The final illustrations were processed using Adobe® Photoshop® CS6 software. The specimens are deposited in the 'National Zoological Collections' of the Zoological Survey of India, Western Ghat Regional Centre, Kozhikode (ZSIK).

***Oxyophthalma engaea* (Wood-Mason, 1889)**  
**(Insecta: Mantodea: Eremiaphilidae)**

Subfamily: Iridinae; Tribe: Dysaulini

Material examined: 1 female, INDIA, Kerala, Thiruvananthapuram, Kerala University,

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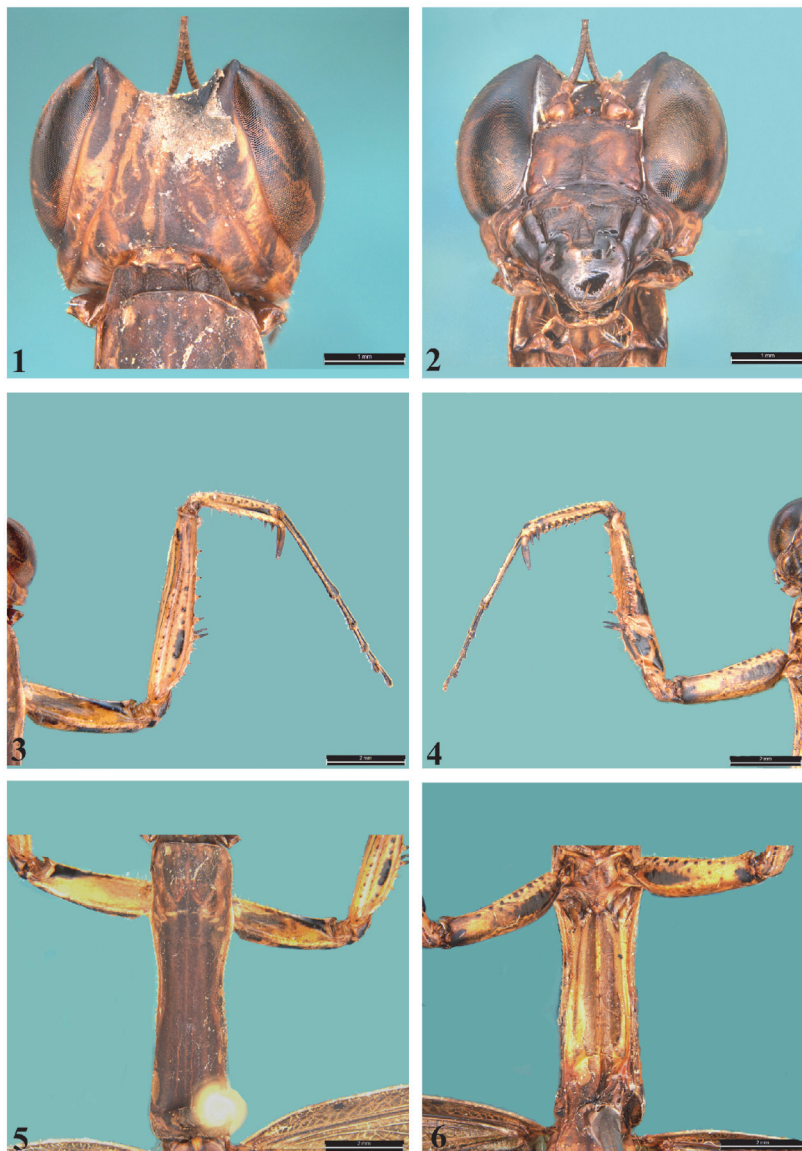


Fig. 1. Head dorsal view, 2. Head frontal view, 3. Foreleg dorsal view, 4. Foreleg ventral view, 5. Pronotum dorsal view, 6. Pronotum ventral view

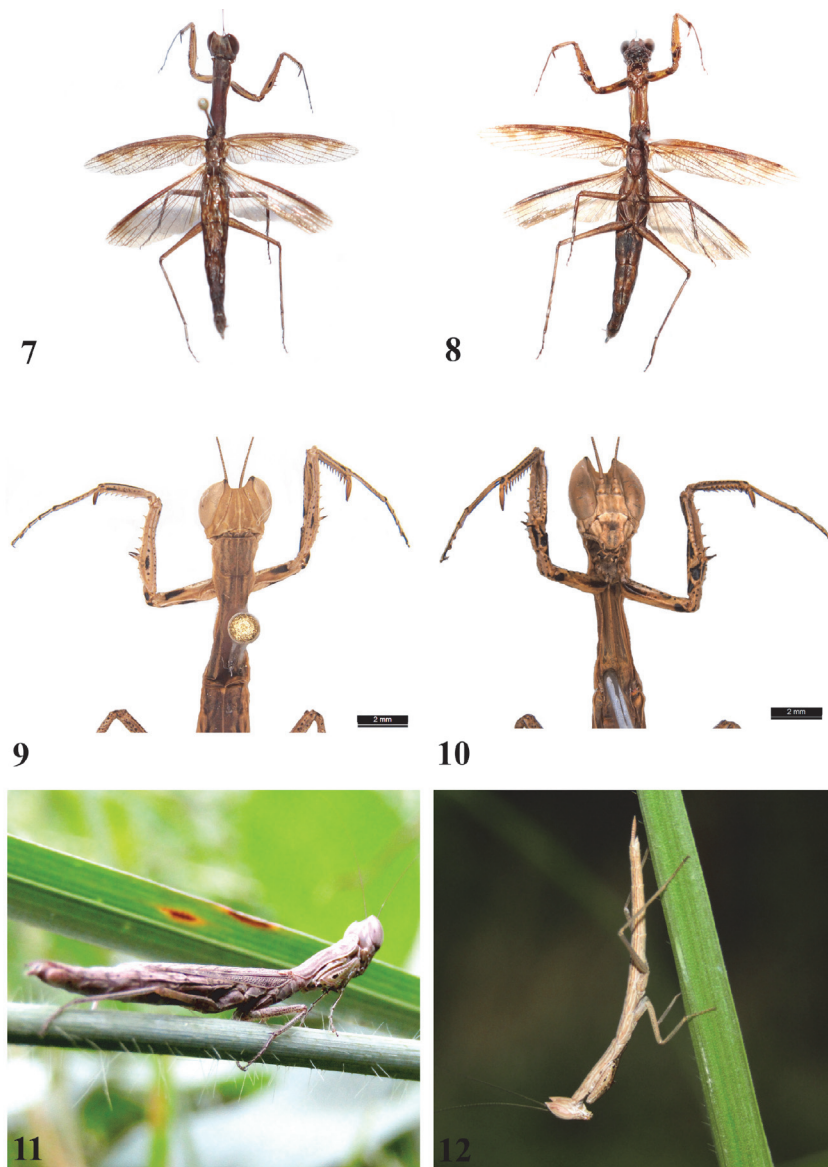
Karyavattom Campus (8° 33' 43" N - 76° 53' 02" E), 27-ix-2019, coll. A.P. Kamila (Reg. No. ZSIK – INV 16946). 1 female nymph, same data as above.

Measurements of adult specimen (mm): Total length 28.5, Forewing 13.98, Hindwing 12.27, Prozona 2, Metazona 6.75, Foreleg: Coxa 6.6, Femur 7.7, tibia 6.3.

Body: Slender, brownish with black spots and patches (Figs. 7-12).

Head: Elongate. Eyes conical and spineless (Fig. 1). Frontal sclerite trapezoid, a little wider than high (Fig. 2). Vertex strongly excavated, with deep brownish patches. Lateral lobes prolonged into a sharp point which extend a little above the upper margin of eyes. Antennae simple.

Pronotum (Figs. 5, 6): Long, slender and rectangular. Supra-coxal dilation not prominent. Pronotum constricted a little after dilation. Disc of pronotum smooth. Metazona about 3 times longer than



7. Adult-body dorsal view, 8. Adult-body ventral view, 9. Nymph-body dorsal view, 10. Nymph-body ventral view, 11. Adult female-live habitus, 12. Nymph-live habitus

prozona. Lateral margins of metazona with small serrations and that of prozona simple. Metasternum with a median ridge and two lateral grooves.

Foreleg (Figs. 3, 4): Yellowish brown with black spots. Fore coxa dorsally with a black triangular patch in the apical region which extends proximally, ventrally with a black line near base and a small patch near apex. Trochanter with a black spot dorsally, ventrally with a small one. Fore femora

with a black line near the base dorsally, ventrally with four black patches (two at base, one in the middle and one along the claw groove). A black patch between the external and discoidal spines. External spines 5, gradually shorter towards distal end; internal spines 14 (8 short and 6 long); discoidal spines 4, first minute, third long and fourth decumbent towards apex. Claw groove situated in the middle of basal half. All fore femoral spines black at apex only, except third discoidal spine. Fore



tibiae with 7 external spines, black at apices; 10 completely black internal spines; internally with a black spot in the apex and externally with a black line which extends from middle to the apex. First tarsal segment almost as long as other segments taken together.

Middle and hind legs: Brownish, with dense black spots. Mid and hind femora with an apical spines. Hind metatarsus shorter than other segments taken together.

Wings (Figs. 7, 8): Both wings shorter than abdomen. Fore wing smoky, hind wing with brown costal area and other areas hyaline.

**Remarks:** *Oxyophthalma engaea* was originally described by Wood-Mason based on three males, two females and four nymphs from Nilgiri hills, South India as *Oxyophthalmus engaeus* in 1889. The genus *Oxyophthalmus* was described by Saussure in 1861, later he renamed it as *Oxyophthalma* in 1869. Nevertheless, *Oxyophthalmus* continued to be used until Giglio-Tos (1927) when Wood-Mason's species name was also corrected to *engaea* (Mukherjee *et al.*, 1992). The present record of this species from plains, Kariavattom, Trivandrum, Kerala is interesting. The species has been reported in India only from higher elevations. It was originally described from Nilgiri hills (elevation 900-2000m) and later reported from Shikharam, Kurnool District of Andhra Pradesh (elevation about 860 m) in 2004 (Rao *et al.*, 2005).

The collected nymph specimen has well-developed wing buds. The spots and patches on the legs of female nymph specimen (Fig. 9, 10) are very similar to that of the female adult specimen. The body colour of the nymph is a little paler than the adult. The deep brownish markings on the vertex of the adult are absent in nymph except on the tip of the extended lateral lobes above the upper edge of the eyes.

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## Influence of humidity on feed utilization of *Cricula trifenestrata* (Helfer) (Lepidoptera: Saturniidae)

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**ABSTRACT:** The wild silkworm (*Cricula trifenestrata*) reared under nutritional humidity and environmental stress condition to determine growth and dietary efficiency, compared with a control indicated that consumption of leaves is significantly influenced by humidity.

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**KEY WORDS:** *Cricula* silk worm, cashew leaf, digestibility, conversion efficiencies

*Cricula* silk moths *Cricula trifenestrata* (Helfer) (Lepidoptera: Saturniidae) are usually seen on wing around August, with a possible second brood from August to September. However, due to drastic climate changes it is getting harder to predict their appearances. Unlike the domesticated *Bombyx mori* silkworm which feeds solely on mulberry leaves, the *Cricula* is a polyphagous. It is capable of feeding on a variety of host plants. In Java, *Cricula* feeds on the leaves of cashew, soursop, avocado and mahogany, with a keen preference towards cashew leaves. The naturally golden cocoons have been successfully utilized into wild silk yarns and other crafts, creating sources of income for local villagers. *Cricula* are utilized for the production of wild silk yarns. *Cricula* is a world intangible cultural heritage but the fabrics used to create this textile art such as silk was too expensive for the low income. Educating the villagers on how to turn the *Cricula* cocoon into wild silk yarns and the business partners who purchase the wild silk

yarns helped to restore the *Cricula*'s natural habitat by planting cashews, avocados etc. The village has evolved into a sustainable village, with higher income and creativity in utilizing their local resources. Cocoon composed of bright golden yellow silk united into a network; the female spins larger cocoon than the male to accommodate its larger size. The golden cocoon is completed in about 8 hrs; this incubation phase lasts for 21-26 days. However, during radical climate condition, it may last for 2-3 months on the Island of Java. With technology, collaboration with the golden cocoons has been utilized into wild silk yarns and other crafts, creating a source of income for local villagers. The newly hatched first instar caterpillars moult and transform into II, III, IV and V instars. In general, the female larva is larger and heavier than males. The male and female larvae can be easily distinguished from its sexual marking. On maturity the larva stop feeding and get start to spinning for the protection of pupa in cocoon shell. Moth

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emerges from cocoon by making a hole in anterior end of the cocoon (Pal and Medda, 2006; Reno et al., 2008). The male moth flies actively after emergence and couples naturally with an available female. Oviposition ranges 200-300 while the same in natural conditions (Kakati and Chutia, 2009; Tikadar, 2012). The male moth survives 4-5 days and female moth for 4-7 day for oviposition of eggs (Yadav and Kumar, 2003; Sarmah et al., 2010).

In the present study this new productive non-mulberry silkworm suitable for rearing during favorable season (August-February) under the Indian condition was taken up. The rearing was conducted as per the new standard package and recommendation by providing fresh leaves of cashew. About 100 larvae in three replicates were separated and reared in humidity and feeding conditions (treatments at 100% RH and at 80-85% RH as control) in sericatron (under normal environmental condition). Cashew leaf used for each feeding was placed in separate trays dummy or dry weight determination of ingested. Additional larval batches of each treatment were maintained in parallel to determine the dry weight and for subsequent determination of daily increment in larval weight (Maynard and Loosli, 1962). The based feeding of wild silk larva is cashew followed by mango, black berry, olive, soalu and som (Rono et al., 2008; Tikader et al., 2010a, 2010b; Tikader, 2011, 2012). The cocoon color varies depending on the host plant, the quality of leaf and its biochemical constituents (Kato et al., 2004). The healthy larvae were counted daily in each replication while the unhealthy and dead larva were removed and replaced from additional batches. The litter was collected carefully on subsequent days of feeding. The excreta and leftover leaf in litter were manually separated and dried in an oven. Observations on III, IV and V instar larval growth were recorded and for dietary efficiency calculation dry weight of left over leaf, excreta, larval weight gain cocoon weight and shell weight and shell weight were recorded for all the replications of each treatment. The experiment was repeated and the data were subjected to statistical analysis to find out the significance. The nitrogen contents of all samples were determined by Micro-Kjeldahl

method. The digestibility and conversion efficiencies were calculated by making use of the following formulae -

Digestibility (%) =

$$\frac{\text{Amount of cashew leaf nitrogen digested}}{\text{Total consumed}} \times 100$$

Conversion efficiency based on consumption (%) (A) =

$$\frac{\text{Increase in dry weight nitrogen of larva}}{\text{Total consumed during that period}} \times 100$$

Conversion efficiency based on digestion (%) (B) =

$$\frac{\text{Increase in dry weight nitrogen of larva}}{\text{Total digested during that period}} \times 100$$

The nutritional indices consumed and dietary consumption of III, IV and V instars are presented in table 1 and 2. The feed consumption is higher for the worms reared under 100% humid atmosphere than for the control for all instars studied (Table 1). It would thus appear that rearing the silkworms under the prevailing conditions of humidity of the atmosphere entails a considerable wastage of the non-mulberry feeds, through the average percentage of consumption for the entire period works out to be the same. Nutritional efficiency in the larval stages significantly influences the resulting pupa, adult and production of silk particularly in an economically important insect like *Bombyx mori* (Takano and Aral, 1978; Aftab Ahmed et al., 1998). The efficiency with which food substances is ingested and converted to larval body matter varied prominently among the hybrids. It was reported that silkworm hybrids were more efficient in converting the food to larval body matter (Trivedi and Nair, 1999; Singh and Das, 1996) reported that less food consumption wild silkworm batches have high efficiency of conversion of ingested and efficiency conversion of digest to cocoon and shell. This may be due to the fact that less choice of feed leads to some physiological adaptations overcomes nutritional stress conditions (Mishra et al., 2011; Keto, 2000). It is clear that the digestibility on the basis of dry weight of leaves remains fairly constant in neighborhood of 35% for the worm reared under the saturated atmosphere in contrast to the widely fluctuating values (nitrogen basis) for the control wild silkworm. Similarly results have

Table 1. Consumption of cashew leaf during different instars (dry weight basis)

Treatment	Total Supplied (g)			Consumed (g)			Consumed (%)		
	III	IV	V	III	IV	V	III	IV	V
100% RH	0.94	4.7	51.7	0.37	2.23	19.7	39.4	47.4	38.1
Control 80-85% RH	1.6	4.9	40.6	0.42	1.7	13.7	20.2	34.2	33.6

Table 2. Cashew leaves consumed to produce unit body weight of different instars (weight of dry leaves in g)

Treatment	III	IV	V
100% RH	4.8	5.5	4.7
Control 80-85% RH	6.3	4.2	9.4

obtained for the conversion efficiencies based consumption (A) and digestion (B) which lie in the vicinity of 20 and 50 per cent for experimental worms.

The data indicate that the more or less constant values (nitrogen basis) for the digestibility and conversion efficiencies for the worms recorded at 100 per cent RH, while it varied in the control (Table 2). These values, it may be observed, are always at a considerably high level due, perhaps to the intense protein metabolism occurring in the silkworms in the humidity chamber (Junliang Xu and Xiaofeng Wu, 1992). Similarly, significant differences of approximate digestibility were observed between all the treatments and controls. Digestibility is affected by nutritional deficiency or imbalanced diet, high content of crude fiber or deficiency of water in food (Waldbauer, 1964; Muniraju *et al.*, 1999). The higher assimilation efficiency or approximate digestibility is certainly a racial character as higher food intake does not necessarily result in higher digestibility (Magdum *et al.*, 1996; Meenaland Ninagi, 1995) reference ratio is an indirect expression of absorption and assimilation of food. It is also expressed in ingest required per unit excreta production. Higher reference ration values mean high rate of digestion and absorption of food. It shows that more food is consumed by the larvae at low humidity in order to build up body weight. This is in accordance with the observation of Singh and

Ninagi (1995), Das *et al.* (1999) and Nath *et al.* (1990) on silkworm food utilization efficiency. This would lead one to that, a part of wild silkworm diet consumed at low humidity serves the purpose of maintaining the water balance that gets disturbed during rearing conditions under varying atmosphere conditions of humidity. In the present study it was clear that larvae growth and nutritional indices parameters were recorded significantly higher when larvae were reared under optimum environmental temperature and humidity and adequate feed quantum as per the recommendation. Though a sericigenous insect, it is now commercially utilized only in Indonesia where it is used as alternative source of income and has become popular among the farmers. With the wide availability of cricula cocoons, the perception of people has also changed and people have started appreciating and enjoying the beauty of its silk. The silk manufacturers like the cocoon due to its unique gloss and gold color, which makes an attractive material for fabrics. The cloth materials produced from cricula cocoons are special due to its strong characters such as water resistance and golden color, cool to wear, heat resistance, non allergenic and anti-bacterial properties. The silk thread of cricula products is lighter and more water absorptive than the normal silk, and has an elegant luster with soft touch. Thus, rearing and conservation of cricula are worthy to include in the activities of the sericulture industry. The insect is capable of surviving and producing cocoons and functional adults at 40°C diurnal temperatures. The genes responsible for such high temperature tolerance need to be elucidated for their possible utilization in developing hardy *B. mori* (Tikader *et al.*, 2013), especially in light of the predicted global warming. The findings demonstrate environmentally induced quality parameters and humidity of cashew leaf that must not be ignored for the successful wild silkworm crop.



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